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A RUST OF THE PIMENTO TREE IN JAMAICA, B. W. I.[1]

J. D. MacLachlan

(Accepted for publication October 30, 1937)

INTRODUCTION

A devastating disease of the pimento tree, *Pimenta officinalis* L., was reported from various parts of Jamaica early in 1934.[2] This disease, apparently new to science, manifested itself by blighting the foliage, young growing twigs, and inflorescences. The causal organism, *Puccinia psidii* Wint., was determined by Miss E. M. Wakefield.

This rust is of interest because it concerns certain products peculiar to Jamaica, namely, allspice, pimento oil, and a liqueur. Furthermore, the host plant belongs to the Myrtaceae, which includes several genera of economic importance. The results of the investigations now presented concern the biology of the causal organism, the factors affecting incidence of the disease, and a discussion of control methods. A preliminary report has been published.[3]

RANGE AND ECONOMIC IMPORTANCE

So far as is known, the occurrence of *Puccinia psidii* as a parasite on pimento is restricted to Jamaica. The disease caused by it made its initial appearance, in proportions to attract the attention of the grower, in April, 1934. The first outbreak occurred in the vicinity of Mandeville, and, within a remarkably short time, had spread throughout the pimento areas at the higher altitudes. Within 2 years the fruit crop in these areas was almost a complete loss, and defoliation necessitated the closing of oil distilleries. In many areas the trees are now either dead or dying from the effects of the disease.

CULTURAL TECHNIQUE

Seedling trees planted in bamboo pots were used for cultural studies in the laboratory. Prevailing high temperature made it necessary to conduct these studies under controlled temperature conditions. Consequently, a culture chamber was devised, capable of providing any desired temperature between 40° F. and 95° F. and maintaining it within 1° F. Moist absorbent

[1] The investigation was made possible by the tenure of a Sheldon Travelling Fellowship from Harvard University. For this appointment grateful acknowledgment is expressed. The writer also wishes to thank Professor J. H. Faull for many helpful suggestions and criticisms. Further appreciation is accorded for the whole-hearted support that has been given, throughout this investigation in Jamaica, by the Director of the Department of Agriculture, the Honorable A. C. Barnes, C. M. G.; the Microbiologist, Mr. F. E. V. Smith, B.Sc.; as well as the agricultural representatives and members of the planting community. All photographs, except figure 1, C, were made by Gick, photographer, Kingston, Jamaica, B. W. I.

[2] Smith, F. E. V. Rust disease of pimento. Jour. Jamaica Agr. Soc. **39**: 408–411. 1935.

[3] MacLachlan, J. D. The pimento rust disease. Jour. Jamaica Agr. Soc. **40**: 277–281. 1936.

cotton was wrapped around the base of the seedling. Spores were scraped from diseased leaves and either shaken up in distilled water and painted on the leaves or else dusted on leaves that had been previously atomized with distilled water. Both methods gave good results. Over the top of the seedling was inverted a small chamber lined with wet filter paper. The seedling was then placed in the culture chamber, and maintained at 60° F. for a period of 2 days, following which the cover and moist cotton were removed. The seedling was then placed in a shaded place in the garden.

Cultural studies in the field involved the following procedure: Sample branches bearing clean foliage were screened within a double layer of cheese-

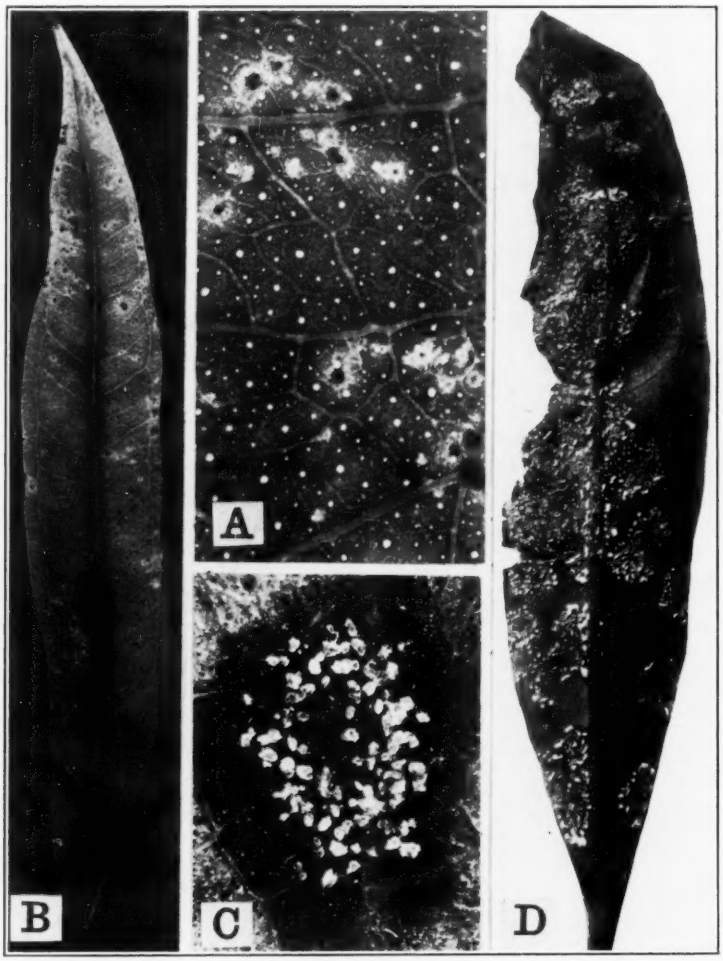

FIG. 1. Two physiological strains of *Puccinia psidii* on rose apple and pimento, respectively. A and B. Rose apple leaf that was inoculated with urediospores from a pimento leaf. This small type of lesion soon dies and never produces urediospores. Portion enlarged in A. C. A uredial lesion on pimento that resulted from inoculation with urediospores from a diseased pimento leaf. Urediospores from diseased rose apple are incapable of infecting pimento. D. Uredial lesions on rose apple leaf that was inoculated with urediospores from diseased rose apple.

cloth for a period of 5 days to eliminate interference by natural infection. Then the screen was removed and the leaves atomized with distilled water, dusted with fresh urediospores, and enclosed for 2 days within celluloid cylinders. The ends of the cylinders were plugged with moist sphagnum, and green leaves were wrapped around the cylinders to prevent direct exposure of the inoculated leaves to the sun.

BIOLOGY OF THE CAUSAL ORGANISM

Biological Specialization

There appear to be 2 distinct strains of *Puccinia psidii* parasitizing rose apple, *Eugenia jambos* L., and pimento, respectively, in Jamaica. Diseased rose apple and pimento, growing side by side, are of common occurrence. Nevertheless, urediospores from diseased rose-apple leaves were found, by inoculation, to be incapable of infecting pimento foliage. On the other hand, urediospores from diseased pimento tissues will form minute lesions (Fig. 1, A and B) on rose-apple leaves. These lesions never sporulate, and, within 2 weeks after their appearance, they turn brown and become necrotic. Similar lesions also were found in the field on rose-apple foliage growing near diseased pimento trees. Urediospores from diseased rose apple and pimento were collected at Stoney Hill, Castleton Gardens, Mavis Bank, New Castle, Content Gap, and within vicinities of Mandeville and Portland. Using the urediospores from these different sources, inoculations were made on healthy seedlings of the 2 hosts. Pimento seedlings were readily infected by urediospores from diseased pimento; the same held true for rose-apple seedlings inoculated with urediospores from diseased rose apple. In all cases, however, when cross inoculations were made, the results obtained were as indicated above.

Seedling inoculations showed that bay rum, *Pimenta acris* Kostel, was susceptible to the strain of *Puccinia psidii* parasitizing pimento, *Pimenta officinalis* (Fig. 2, B). Urediospores from the diseased bay-rum foliage were used to inoculate pimento and rose-apple seedlings. The pimento seedlings were readily infected, while the rose-apple seedlings exhibited the same type of lesion that was obtained when the inoculum from diseased pimento was used. On the other hand, the otaheite apple, *Eugenia malaccensis* L., was susceptible to the strain of *P. psidii* parasitizing rose apple, *Eugenia jambos* L. (Fig. 2, A). Urediospores from diseased otaheite-apple foliage readily infected rose apple, but were incapable of infecting pimento.

Puccinia psidii was originally described on *Psidium pomiferum* L. in Brazil by Winter.[4] *Psidium guajava* L., the common guava, was recorded as a host of this rust in Puerto Rico by Arthur.[5] According to Popenoe,[6]

[4] Winter, G. Rabenhorstii fungi europaei et extraeuropaei. Cent. XXXI. et XXXII. Hedwigia **23**: 164–175. 1884.

[5] Arthur, J. C. Uredinales of Porto Rico based on collections by F. L. Stevens. [Numbers 36–75.] Mycologia **7**: 227–255. 1915.

[6] Popenoe, W. Manual of tropical and subtropical fruits. . . . 474 pp. Macmillan Co., New York. 1920. (Reprinted 1924, 1927, 1934.)

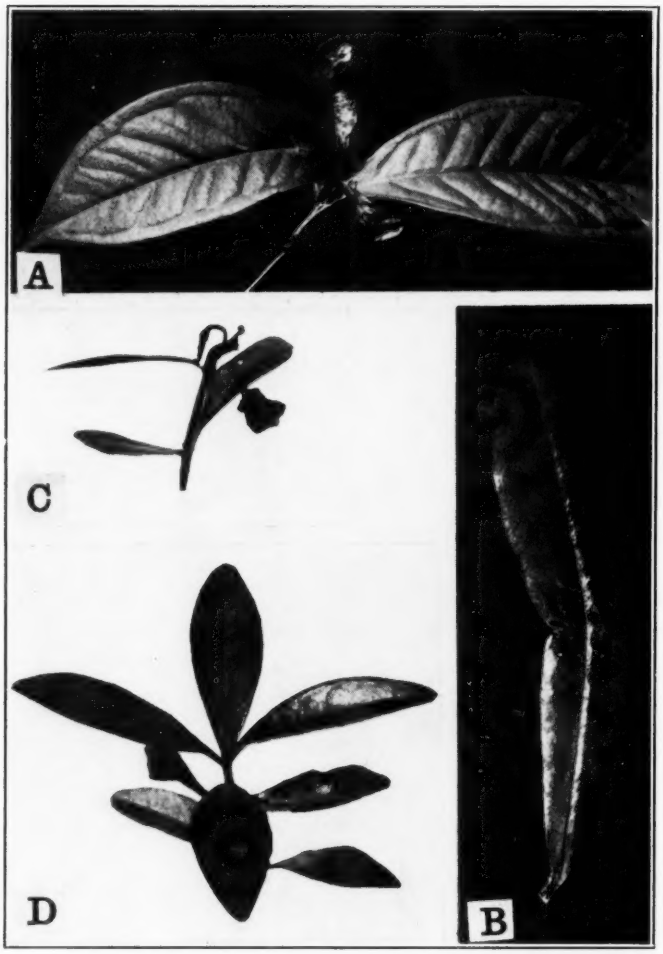

FIG. 2. A and B. Two hosts of *Puccinia psidii* hitherto unreported. A. Diseased seedling of the otaheite apple (*Eugenia malaccensis*) infected by urediospores from diseased rose apple. No infection could be obtained on the older leaves. B. Diseased leaf of the bay rum (*Pimenta acris*) infected by urediospores from diseased pimento. Note the distortion from a single lesion. C and D. Effect of temperature on the incidence of the disease caused by *P. psidii*. C. Seedling prepared as that in D but kept at an estate above 1,000 feet altitude for two weeks. One diseased leaf dropped; all new leaves were killed from severe infection. D. Seedling bearing two diseased leaves was kept at sea level for two weeks. All new leaves were healthy.

p. 275, *Psidium pomiferum* is a variety of *P. guajava*. Guava trees are of common occurrence in Jamaica within regions where *Puccinia psidii* is prevalent on both rose apple and pimento. No natural infection of guava by this rust fungus was observed. Guava seedlings proved to be immune when inoculated with urediospores from diseased pimento, bay rum, rose apple, and otaheite apple, respectively. It would appear then, that the two strains of *P. psidii* now existing in Jamaica are physiologically, at least, different from the strain from which the original description was made.

Hosts and Their Relative Susceptibility

The strain of *Puccinia psidii* parasitizing species within the genus *Pimenta* is apparently more virulent with respect to its effect on the hosts than the strain parasitizing species within the genus *Eugenia*. The hosts of the 2 strains of *P. psidii* that are found in Jamaica and their relative susceptibility may be summarized as follows:

A. Hosts of the strain of *Puccinia psidii* parasitizing species within the genus *Pimenta*.
   Pimenta officinalis L. Very susceptible. Determined by inoculation and from natural infection.
   P. acris Kostel. Moderately susceptible. Determined by inoculation and from one diseased seedling found at Mavis Bank.
   Eugenia jambos L. Resistant; lesions never sporulate and soon die. Determined by inoculation and from natural infection.
   E. malaccensis L. Resistant; lesions formed similar to those on *E. jambos*. Determined by inoculation.
   Psidium guajava L. Immune. Determined by inoculation and by field observations.

B. Hosts of the strain of *P. psidii* parasitizing species within the genus *Eugenia*.
   Eugenia jambos. Very susceptible. Determined by inoculation and from natural infection.
   E. malaccensis. Moderately susceptible. Determined by inoculation.
   Pimenta officinalis. Immune. Determined by inoculation.
   P. acris. Immune. Determined by inoculation.
   Psidium guajava. Immune. Determined by inoculation and by field observations.

Symptomatology

The urediospores of *Puccinia psidii* are capable of infecting the young growth only of pimento. This includes the expanding foliage, the inflorescences and the succulent young twigs. Figure 3 illustrates symptoms and signs of the rust. Diseased tissues are initially manifested by the bright yellow, powdery masses of urediospores. On the foliage these spores usually are formed on the lower surface of the blade, sometimes on the upper. Light infections appear as scattered lesions varying in size from pin-point to more than a centimeter in diameter. If the infection is severe the lesions coalesce, so that the entire leaf may be covered with a mass of urediospores. The pathogen may invade any portion of the cymose inflorescences. In many cases, infection of succulent twigs appears to be systemic, as the entire new growth of a twig may exhibit urediospores (Fig. 3, A). This phenomenon may be due to the natural growth of the twig after infection has taken place.

Light foliar infection, where only scattered lesions exist, will not cause material harm. Some distortion may occur but the leaves persist for their normal life period. After the spores have been shed the lesions turn brown to black, with scarcely any evidence of rust pustules. Severe foliar infection results in death to the leaves and defoliation within a short time. Figure 3, A and B, illustrate the effect on the foliage of various degrees of infection. Premature defoliation results in the production of new foliage that in turn becomes infected. A continuance of successive attempts to produce foliage soon utilizes the reserve energy of the tree culminating in its death. During the first season following severe infection, the effect on the foliage

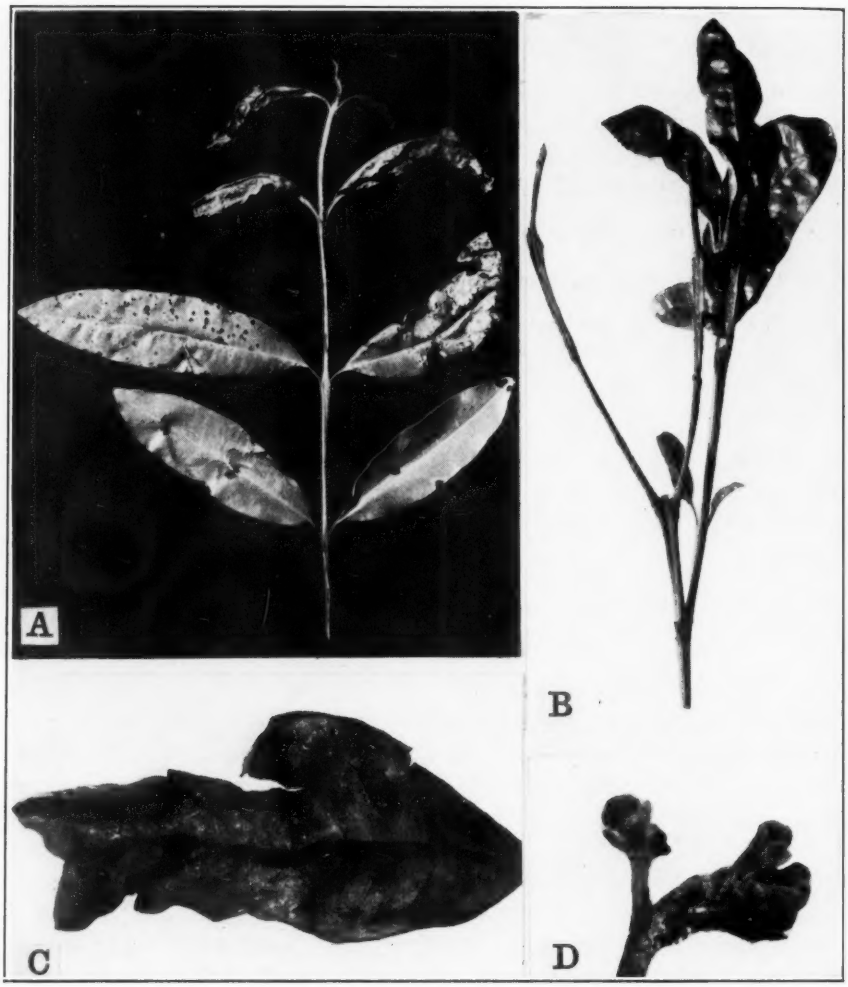

FIG. 3. Symptoms of the disease caused by *Puccinia psidii* on pimento. A. Diseased twig that illustrated various degrees of infection. The lower pair of leaves represented the type that is immune from new infections. Beyond the second pair of leaves the twig was covered with urediospores. B. Twigs that had been defoliated and killed by severe infection. C. A diseased leaf that exhibited both urediospores and teliospores. The distal half of the blade was dying. Urediospores show white in the photograph; teliospores were present at the centers of the lesions and were very abundant along the curled dead portions. D. Diseased portion of an inflorescence. Urediospores were exhibited on all portions.

is not so apparent, because the immune, one-year-old leaves still persist; these leaves, however, normally drop 2 years after their formation.

Infection of the inflorescences causes them to turn black and die. Diseased blossoms usually drop before the fruit has set. A single lesion on a pedicel is sufficient to cause that blossom to die. Infected young fruits may persist for a short time but they soon shrivel, turn black, and drop.

Infected twigs usually die because of the systemic nature of the rust within them (Fig. 3, A and B). Trees that have been subjected to severe

infection for more than a year become so weakened that twig development ceases.

Life History

Puccinia psidii apparently survives, in Jamaica, by means of the urediospore cycle only. An intensive search revealed no host for the haploid stage. The urediospore cycle involves a period of approximately two weeks. Ten to 12 days after inoculation small, watery, yellowish blisters appeared on the dorsal side of the inoculated leaves. Within 2 days these blisters erupted and exposed the bright yellow uredia. The duration of urediospore formation varies. Severe infection causes diseased leaves and blossoms to drop within 3 to 4 weeks; severely diseased twigs usually die within the same time limit; and in such cases the fungus dies with the diseased host organ. Scattered foliar lesions may cease to exhibit urediospores at their centers but continue to expand and produce them at their peripheries (Fig. 1, C) for a period of at least one month. After all the urediospores have been shed the lesions are smooth and greyish brown. They may remain in this condition for months without exhibiting any evidence of teliospore formation. Old lesions appear brownish black and are apparently necrotic.

Teliospores were very abundant on diseased pimento foliage during October and November, 1935, but were of rare occurrence during the remainder of the writer's tenure in Jamaica, namely, until April 15, 1936. Figure 3, C, illustrates a pimento leaf that is bearing urediospores on the periphery of lesions within the living portions of the leaf, and a great abundance of teliospores on old uredia within the apparently dead portions of the leaf.

FACTORS AFFECTING THE INCIDENCE OF THE DISEASE

A. EXPERIMENTAL DATA

Duration of Susceptibility of Host Tissue

Very young as well as dark green mature leaves of a pimento tree in the field were inoculated with urediospores from diseased pimento foliage. The young expanding leaves became severely diseased; the mature leaves exhibited no evidence of infection.

Leaves of various known ages on seedling pimento trees were inoculated with urediospores from diseased pimento foliage. The age of a leaf was considered to be the lapse of time between the parting of a pair of the new leaves concerned and inoculation. Data on the results obtained are given in table 1. The new leaves showed considerable variation in expansion and maturation. The majority of them ceased to expand and turned dark green 4 to 5 weeks after parting; these leaves were then immune. Others continued to expand to abnormal size and remained light yellowish green; these continued to be susceptible, but no infection was obtained on any leaves that were more than 45 days old.

TABLE 1.—*Duration of the period of susceptibility of the leaves of pimento seedlings*

Age of inoculated leaves in days[a]	Number of seedlings inoculated	Number of seedlings infected
0	8	8
20	3	3
22	3	3
24	3	3
28	3	2
32	3	3
34	3	2
35	3	2
36	3	2
37	3	0
38	3	1
40	3	1
42	3	0
45	4	1
50	3	0
53	3	0
60	7	0

[a] Leaves of seedlings that were more than 37 days old at the time of inoculation were but slightly infected. Relative to the average leaf, these leaves had grown to abnormal size and were immature when inoculated.

Relation of Time in Culture Chamber to Infection

An experiment was conducted to determine the length of time necessary for the inception of infection. The young leaves on 34 pimento seedlings were inoculated and placed in the culture chamber. At intervals of 2, 4, 6, 8, 10, 12, 14, 16, 19, 21, 24, 27, 30, 33, 36, 39 and 42 hours, respectively, two seedlings were removed from the culture chamber and exposed to a temperature of more than 75° F. where the surfaces of the inoculated leaves dried within a few minutes. Observations were made 2 weeks later. No infection was exhibited by any of the seedlings removed from the culture chamber in less than 14 hours. The seedlings incubated for 14 hours showed a few scattered lesions. A progressive increase in the number of lesions per leaf was exhibited by the seedlings incubated for 16 and 19 hours, respectively. Incubation periods of more than 19 hours resulted in no further increase in the amount of infection.

Duration of Viability of the Urediospores

Urediospores of a known age and in sufficient quantity for an experiment to determine the duration of their viability were not available. Therefore, spores were scraped in large quantity from newly formed lesions on diseased pimento foliage and mixed thoroughly. Samples of these spores were placed on clean cover glasses. Equal numbers of the prepared cover glasses were arranged in 3 separate wire trays. One tray was suspended from the roof inside a small pump-house where the daily temperature was well over 90° F. The 2 remaining trays were placed in the culture chamber at 60° F., one within a desiccator. At intervals of 2 to 3 days, 2 cover slips were removed

from each tray and the spores on each cover slip were used to inoculate individual seedlings. The viability of the spores at the time of inoculating was indicated 2 weeks later by the presence or absence of lesions on the inoculated seedlings (Table 2).

TABLE 2.—*Duration of the viability of urediospores of P. psidii when subjected to different temperature and humidity conditions*

Time interval in days between preparation of spores and inoculation	Indication of the presence (+) or absence (0) of infection resulting from urediospores that had been kept in		
	Pump house (high temp.)	Culture chamber at 60° F.	Desiccator at 60° F.
1	+	+	Seedlings dead
2	+	+	+
4	+	+	+
5	+	+	+
7	0	+	+
9	0	+	+
11	+ very slight	+	+
14	0	0	+
16	0	+	0
19	0	0	0
22	0	+	+

Temperature Relationships

The significance of temperature and its correlative altitude on the incidence of the disease was illustrated by the following series of experiments in which susceptible young leaves of pimento seedlings were inoculated with urediospores. Six seedlings were used in each unit.

(1) Seedlings were inoculated and immediately placed in a garden at sea level where the prevailing temperatures were more than 75° F. No infection was obtained.

(2) Inoculated seedlings were incubated for two days at 60° F., then placed in the garden at sea level. Severe infection was observed on all seedlings two weeks later.

(3) Seedlings were taken to an estate of more than 1,000 feet altitude and inoculated. A maximum and minimum thermometer showed that the temperature dropped to 65° F. during the first night of incubation. Severe infection was observed 2 weeks later.

(4) The diseased seedlings of experiments (2) and (3) were used in this instance. One half of the seedlings in the garden at sea level were taken to the estate at an altitude of more than 1,000 feet. Similarly, one half of the seedlings at this estate were taken to the garden. Further data were recorded from these diseased seedlings 2 weeks later. No spread of infection was observed on the seedlings kept in the garden at sea level. The diseased leaves were almost dead but new leaves developed and remained healthy. A different picture was exhibited by the diseased seedlings left on the estate above an altitude of 1,000 feet. As new leaves

were formed at the growing point, they became heavily infected by the urediospores from diseased leaves. Figure 2, C and D, illustrates the results obtained.

An experiment was conducted to determine the range of temperatures within which infection might be obtained in the culture chamber. Seedlings were inoculated, then maintained in the culture chamber at temperatures that varied by approximately 5° F. intervals and ranged from 40° to 90° F. The experiment was performed in duplicate, and a parallel series of inoculated seedlings was maintained at 60° F. for check purposes. No infection was obtained below 47° F. Repeated and heavy infections occurred between 55° F. and 70° F. Temperatures above 70° F. inhibited infection and no infection was obtained above 80° F. All of the check seedlings exhibited heavy infection.

DISCUSSION

With Respect to the Host

The foliage is most susceptible during the period of expansion but apparently immune when the leaves have ceased to expand and have turned dark green. Whether or not the new leaves on a tree in the field will mature and become immune within the time limit exhibited by the seedlings (4 to 5 weeks) may be open to question. It is possible that under field conditions the new leaves would mature even more rapidly than those on seedlings kept under environmental conditions conducive to vigorous growth. There is some variation in the time limit of foliar maturation; field observations substantiated those made on the seedlings. This may explain, in part, the difference in the degree of infection found on the foliage of trees within the same locality. No detailed studies were made on the duration of susceptibility of inflorescences and twigs, but both are very susceptible during their earlier stages of development.

The incidence of the disease is influenced by the prevalence of susceptible host tissue. The presence of seedlings that bear large amounts of succulent growth favors the spread of the pathogen. In pimento areas that have been severely infected for some time, the trees are so weakened that there is little new growth for the pathogen to invade. At lower altitudes, where the degree of infection is less severe, the disease is most apparent in the spring when the new foliage, inflorescence, and succulent young twigs, are normally produced.

With Respect to the Pathogen

Even under optimum temperature conditions the urediospores require a period of 14 to 19 hours in a moist chamber to infect inoculated seedlings. This time limit may, in part, explain the greater prevalence of the disease found in valleys, where mists were frequently observed following a rain, and on hillsides with a northern or western exposure not directly exposed to the morning sun.

Urediospores can be found on diseased pimento foliage at any time of year but they are less abundant during the dry winter months, when little or no new growth is normally produced. The possibility of urediospores overwintering is open to question. They apparently lose their viability within a week when subjected to a temperature above 90° F. It would seem, however, that desiccation does not materially affect the viability of urediospores when subjected to a temperature of 60° F. for 3 weeks.

With Respect to Environment

Humidity. Extended periods of wet weather favor the spread of the disease. It is possible that the prevailing high humidity in 1933 favored the sudden outbreak of the rust in the spring of 1934. During 8 months (June, 1933, to March, 1934, inclusive) the rainfall varied from 50 per cent above normal to 6 times normal. The long periods of wet weather favored infection and contributed to an excess of succulent new growth. A sufficient spore population was established to cause a degree of infection that resulted in defoliation. New leaves were then formed and they, in turn, became infected. In this way a constant source of susceptible host tissue was provided for the pathogen.

Temperature. Pimento is grown from sea level to altitudes of more than 3,000 feet, and a wide range of temperatures naturally prevails. Inoculated seedlings, subjected to various temperatures in the culture chamber, indicated that the temperature range within the pimento areas is a deciding factor in the incidence of the disease. Failure to obtain infection on the inoculated seedlings below 47° F. is not significant, as such low temperature never occurs in the pimento areas. Temperature between 55° F. and 70° F. favors infection and is common at the higher altitudes. Infection is inhibited above 70° F. and such temperatures prevail at sea level.

Preliminary field surveys indicated that the disease was more prevalent at the higher altitudes. This phenomenon was substantiated by keeping inoculated seedlings (a) on an estate of more than 1,000 feet altitude and (b) at sea level. A questionnaire, requesting information concerning altitude of property, annual crop yield, and severity of the disease was sent to pimento growers. Significant replies, which gave a fair representation of the pimento areas, were received from 82. The results (Table 3) indicate that the severity of the disease is directly correlated with altitude. Below 1,000 feet, 99 per cent of the pimento was reaped where the pathogen was either absent or doing no material harm. Between 1,000 and 2,000 feet, 95 per cent of the pimento was reaped where the degree of infection was reported as mild or moderate. At altitudes of more than 2,000 feet, severe infection was reported in all cases. The severity of the disease within the 3 respective altitude limits is reflected in the crop yields of 1934 and 1935. It is also of interest to note that a relatively large percentage of the pimento is collected below an altitude of 1,000 feet, more than 40 per cent during the 4 years prior to the advent of the pathogen and more than 60 per cent afterwards.

TABLE 3.—*Data on crop yields of pimento and relative severity of the pimento rust at respective altitudes in Jamaica*

Altitude of estates in feet	Total bags collected 1930–35	Annual number of bags[a] collected						Per cent of the total number of bags collected from		Per cent of the number of bags collected in 1934 and 1935 where rust infection[b] was considered in 1936 as			
		Before advent of rust				After advent of rust							
		1930	1931	1932	1933	1934	1935	1930–33	1934–35	None	Mild	Moderate	Severe
Sea level to 1,000	13,739	2144	2366	2059	1306	4426	1438	43.3	63.6	74.6	24.2	1.2	0.0
1,000–2,000	9,984	1888	1953	1754	1678	2143	568	40.0	29.4	1.0	25.1	70.2	3.7
2,000 up	3,658	1254	675	627	462	605	35	16.7	7.0	0.0	0.0	0.0	100.0

[a] One bag contains approximately 160 pounds of the dry pimento berries.

[b] The degrees of infection were defined as:

None—No rust.

Mild—Rust is present but not doing any damage.

Moderate—Leaves infected but not killed; crop yield of one half to three quarters of the average.

Severe—Majority of trees defoliated by the rust. Fruit yield almost a total loss.

CONTROL

The feasibility of using fungicides to control the pimento rust is questionable. Most of the trees grow where it would be almost impossible to take a power sprayer. Furthermore, pimento is an extremely variable crop. Even weather conditions during blossom time seem to determine whether 200 or 20 bags will be collected. Thus, it would be difficult to convince the pimento grower to go to the expense of spraying before the fruit has set. In certain instances it might be of value to spray at intervals for 2 months during blossom time. In so doing, protection could be afforded the fruit as well as sufficient foliage to carry the tree through the year.

Although no artificial means of control was found, the pimento industry seems by no means doomed. A relatively large percentage of the pimento is grown at the lower altitudes, where the prevailing high temperature has thus far checked the spread of the disease. In these localities the following suggestions may be of value: 1. Some infection may be found in winter or early spring on the foliage of young and seedling trees. This probably will die out with the advent of warmer weather; but there is some danger of blossom infection, hence the diseased branches should be removed and burned. At least 2 inspections should be made, the first about 2 or 3 weeks prior to blossom time and the second 2 weeks later. This procedure has been tested and proved to be practical as well as efficient. 2. Inquiries have been made with respect to the advisability of planting pimento seedlings at the lower altitudes. This may be a worthwhile project, but should not be attempted until the pathogen, in its ability to spread, has come to an equilibrium with the temperature factor. This should take place within 2 to 3 years.

SUMMARY

A sudden outbreak of *Puccinia psidii* Wint. on the pimento tree, *Pimenta officinalis* L., occurred in Jamaica, B. W. I., in the spring of 1934. Within 2 years, the damage at the higher altitudes was so great that the products derived from the tree were almost a complete loss.

There appear to be 2 distinct strains of *Puccinia psidii* parasitizing pimento and rose apple, *Eugenia jambos* L., respectively, in Jamaica. *P. psidii* was originally described on *Psidium pomiferum* L., a variety of the common guava, *Psidium guajava* L. The latter tree has been reported as a host to *P. psidii* in Puerto Rico, but is apparently immune from the 2 strains of *P. psidii* found on pimento and rose apple, respectively. The bay-rum tree, *Pimento acris* Kostel, is susceptible to the strain parasitizing pimento, and the otaheite apple, *Eugenia malaccensis* L., is susceptible to the strain parasitizing rose apple.

Puccinia psidii attacks the expanding foliage, the inflorescence, and the succulent young twigs. Defoliation results in the development of new foliage that in turn becomes diseased. The fungus is perpetuated in Jamaica in the urediospore stage; teliospores were found, but no host for the haploid stage was recognized.

The incidence of the disease caused by *Puccinia psidii* on pimento is influenced primarily by 1, the relative abundance of susceptible new growth; 2, the existence of long periods of wet weather; 3, temperature. Temperature is the limiting factor that may determine the future of the pimento industry. A relatively large proportion of the pimento is reaped below an altitude of 1,000 feet, where *P. psidii* is either absent or doing no material harm because of the prevailing high temperature, which inhibits infection.

Use of fungicides to control the rust seems impracticable. Suggestions with respect to slight infections taking place at lower altitudes during winter and to the advisability of planting pimento seedlings at the lower altitudes have been given.

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THE RELATION OF CONCENTRATION OF FUNGICIDES AND BUD DEVELOPMENT TO CONTROL OF PEACH LEAF CURL[1]

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INTRODUCTION

The early discovery of the ease with which peach leaf curl (*Exoascus deformans* (Berk.) Fckl.) could be controlled by an application of a strong fungicide during the dormant period of the tree resulted in a rather general neglect of investigational effort to establish the minimum strengths of fungicides necessary to control the disease, and to determine the relation of bud development of the tree to infection and control. The accepted spray practices continue to give satisfactory results, but recommendations have not always been made with due consideration of the practical aspects of the possible monetary savings from the use of weaker sprays and of the fact that seasonal and orchard conditions frequently render spraying impossible until after the buds are active.

When used at the rate of 1 part in 8 of spray, lime sulphur gives good control of both San José scale (*Aspidiotus perniciosus* Comstock) and peach leaf curl, but the use of this material is not necessarily the most economical way to accomplish these results. For example, oil emulsion is a more efficient scalecide and Bordeaux mixture is usually a cheaper fungicide than lime sulphur. Furthermore, lime sulphur will control only San José scale and will not affect the terrapin scale (*Lecanium nigrofasciatum* Pergande) which is frequently troublesome in peach orchards. An oil spray must be used to control this insect and a fungicide may be added to make a complete insecticide-fungicide combination.

[1] Paper No. 87 from the Section of Botany and Plant Pathology, Virginia Agricultural Experiment Station.

REVIEW OF LITERATURE

The life history of the peach leaf curl fungus was poorly understood for years, but it has recently been cleared up by the studies of Fitzpatrick (5) and Mix (9). The incomplete knowledge of the life history of the fungus led to the recommendation of control measures that were too dogmatic in nature.

The more or less general prevailing opinion is well expressed by Zeller (17) who writes, "The exact and very earliest time when this fungus infects the tiny leaves or twigs in the spring of the year is really unknown . . ." and that "Some infection takes place however as soon as the buds swell enough to cause the bud scales to slide over each other and to break the coating of wax which more or less seals the scales together, thus exposing the least bit of leaf tissue or green bases of the scales." Zeller recommends the application of a 6–6–50 Bordeaux mixture after the leaves have fallen and not later than December. This recommendation takes in consideration the problem of controlling California blight (*Coryneum beijerinckii*) as well as that of leaf curl. Heald (7, p. 467) states that spraying after the buds have swollen does not prevent the disease, and recommends that the orchardist "Spray *once*, either in the late fall, early winter or in the spring *before the buds start to expand*. . . ." Pierce (11) states "That most of the spores of *Exoascus deformans* enter upon the stage of germination at or about the time of pushing of the first leaf buds in the spring admits of little doubt. That is the time when the tissues of the peach leaf are most tender, and when their infection by curl is actually known to take place." He recommends that the tree be sprayed 2 or 3 weeks before growth begins, as the spores at that time are just beginning to germinate and are most susceptible to the action of fungicides. He did not consider the matter of later spraying.

Results of early work by various investigators indicated that spraying after the buds had swollen, or even made conspicuous growth, frequently gave successful control; but, despite this fact, the impression that spraying at such a time is always useless became widespread and rather generally accepted. Murrill (10) as early as 1900 recommended that the spray be applied " . . . about the first of April when the buds are beginning to swell." Laidlaw and Brittlebank (8) also recommended that the spray be applied "Just before or when the earliest buds are showing pink. . . ." This recommendation was based upon their investigations in which applications at such a time had been successful. Wallace and Whetzel (14) stated that an application to be effective, if not made before the buds had swollen, could still be made if applied before there was rain favorable to infection. A few years later Reddick and Toan (12) obtained good control in New York State rather late in April and when, in some orchards, the leaves were ½ inch long. No rains favorable to infection had occurred prior to that time, however, and when they set in on April 20, infections apparently developed, as control was not secured with applications made on April 26. Allen (1) in 1918 reported, fair control from applications made when the buds were

"at the pinking stage" although better control was obtained with earlier applications. A few years later Allen and Arthur (2) reported that results from spraying swollen buds were as good as those from buds sprayed while dormant. Weldon (15) obtained good control on the Simms variety in California, sprayed when "practically one half in bloom." Fitzpatrick (5) recently reported good control resulting from applications made when the leaves were fairly well out. He found time of infection to be correlated with periods of rainfall, confirming the observations of Wallace and Whetzel (14) and others.

The value of lime sulphur as a fungicide for the control of peach leaf curl was accidentally discovered in California at the time when this material was first being used as an insecticide to control San José scale. The spray used was necessarily quite concentrated, and, although it was recognized a number of years ago that somewhat weaker dilutions could be satisfactorily employed, no particular attempt was made to ascertain how much lime sulphur might be diluted and yet remain dependable. The tendency to recommend strong spray solutions has persisted in the absence of satisfactory experience with the weaker concentrations.

Pierce (11) used a lime sulphur made from a 5–5–45 formula, which, according to tests made during the current study, corresponds to approximately a 1 in 16 dilution of 32° Baumé lime sulphur, or about half the usual winter strength. Murrill (10) recommended a 6–4–50 Bordeaux mixture for dormant application and a 2–2–50 formula to be applied after the petal fall in wet seasons. Shortly afterward, Farrand (4, pp. 17–18) reported 2 pounds of copper sulphate in 50 gallons of water to make an effective spray. Laidlaw and Brittlebank (8) obtained poor control with lime sulphur, although Bordeaux mixture proved more satisfactory. Their best results were obtained with copper acetate and copper soda solutions. They recommended a 6–8–40 (6–8–50 U. S. standard) copper soda spray as being the most satisfactory, and yet economical. Wallace and Whetzel (14) in 1910 stated that "The fungicide used and the strength of it are of relatively little importance." They found a Bordeaux mixture as weak as 3–4–50 to control curl, and 32° lime sulphur diluted 1–20 to give fair control. Weldon (15), in reporting on comparative tests with dry and liquid lime sulphurs, stated that liquid lime sulphur, as weak as 1–30, and dry lime sulphur at 2 pounds in 30 gallons gave fair control of leaf curl, although somewhat better results were obtained with higher concentrations of each material. The dry and liquid forms proved equal in all tests when used at the rate of 2 pounds of dry material as the equivalent of 1 gallon of liquid lime sulphur. It should be noted that these applications were made when the trees were in almost semi-anthesis, much later than is generally recommended for leaf-curl control.

A year later Allen and Arthur (2) reported satisfactory control with lime sulphur in dilutions ranging from 1–7 to 1–12, with Bordeaux mixture at 4–6–22 (4–6–27½ U. S. standard) and with Burgundy mixture. Forma-

lin, used at the rate of 1½ ounces per gallon of spray, gave no control, nor did sulphur dust. The lime sulphur tested about 25° Baumé, which would make their dilutions correspond to approximately 1–9.7 and 1–16.7 of a 32° solution.

Wilson (16) was able to control leaf curl with fall applications of Bordeaux mixture as weak as 2–5–50, with lime sulphur 4–50 and with basic copper sulphate at 3 pounds in 50 gallons. Copper ammonium silicate (Coposil) at 3 pounds in 50 gallons of spray did not give as satisfactory control.

Stanley, Marcovitch and Andes (13) obtained control with wood creosote when used alone at 8 per cent strength and also when used at 1 per cent strength with 3 per cent oil emulsion.

Cation (3) states that lime sulphur 1–20 is the standard spray dilution used in Michigan, although some growers use a stronger spray. It is probable that very much the same situation exists in most peach-growing States. Zeller (17) recommends a 6–6–50 Bordeaux mixture for the control of both peach leaf curl and California blight.

METHODS AND MATERIALS

Peach trees known to be generally affected with leaf curl were selected for the spray experiments. The trees had in most instances been left entirely unsprayed for all or the greater part of the life of the orchard.

The plots where the various materials and dilutions of materials were under test were sprayed with a power-spray rig at 325 pounds pressure, multinozzle spray brooms being used for application. The individual trees or small plots were sprayed with a knapsack sprayer at 75 pounds pressure.

The plots were examined and the results determined after the trees were in full leaf and before the curl-affected leaves had begun to fall. The number of leaf clusters containing diseased leaves were counted, no attempt being made to count all of the disease-free leaves or leaf clusters. The trees selected for the experiments were for the most part fairly uniform in size, and the counts that were made gave a good indication of the degree of control obtained. Had the results been less clearly evident, it might have been necessary to count the actual number of infections per thousand leaves.

EXPERIMENTS IN 1933–34

Fall applications of various fungicides were made on 7-year-old Elberta peach trees in the Stephenson orchard near Winchester, Virginia, on November 21, 1933. The same treatments were repeated, with a few omissions, on an adjacent block on March 17, 1934. The plots were of 10 trees each. The fungicides employed, their rates of application, and the leaf-curl counts are given in table 1.

All materials used in 1933–34 gave satisfactory control of leaf curl at the several strengths or dilutions tested. The slight variation from plot to plot is not considered significant in view of the difficulty of completely

TABLE 1.—*Efficacy of various fungicides in the control of leaf curl, 1933–34 applications*

Plot No.	Materials and amounts used per 100 gallons	Diseased clusters per plot	
		Fall spray	Spring spray
1	Lime sulphur, 2 gallons	20	22
2	Lime sulphur, 4 gallons	11	0
3	Lime sulphur, 6 gallons	0	1
4	Lime sulphur, 9 gallons	1	0
5	Lime sulphur, 12 gallons	0	9
6	Dry lime sulphur, 16 pounds	0	Omitted
7	Dritomic sulphur, 16 pounds	58	2
8	Dry flotation sulphur, 16 pounds	4	Omitted
9	Soluble sulphur, 8 pounds	0	6
10	Soluble sulphur, 16 pounds	0	Omitted
11	Kolofog, 16 pounds	11	16
12	[a] Bordeaux, 2– 4–100 formula	57	30
13	[a] Bordeaux, 4– 8–100 formula	6	0
14	[a] Bordeaux, 6–12–100 formula	1	0
15	[a] Bordeaux, 8–16–100 formula	0	Omitted
16	Check, nonsprayed	1859	1858

[a] All Bordeaux mixture was prepared with a special grade of hydrated spray lime and in accordance with common practice.

covering all of the surface of 10 trees, and of the possible occasional infection resulting from conidia carried by wind or rain from adjacent nonsprayed trees. The plots sprayed with 2–4–100 Bordeaux showed slightly more leaf curl for both the fall and spring treatments than did those sprayed with the stronger combinations. The difference was only slight, however, and was but 2.4 per cent of the infection on the checks.

EXPERIMENTS IN 1935

The further testing of numerous fungicides at varying strengths was not continued in 1935 because of the favorable results obtained in the 1933–34 season and the decision of the owner of the orchard in which the experiments were being conducted to do the spraying himself.

Appreciation of the fact that only a very few growers complete, and that many frequently do not begin the leaf-curl spray prior to the swelling of the buds, led to the trial of a delayed spray application in 1935, which in timing paralleled that of many growers. Fifteen trees were sprayed in the Stryke orchard, a small home planting near Winchester. One spray was applied on March 18, 1935, when the young leaves were protruding about $\frac{1}{8}$ inch from the buds. There was a rather uniformly distributed rainfall of 1.64 inches for the 3-day period, March 11, 12, and 13, four days before the spray was applied. Since the peach buds were conspicuously swollen at the time these rains occurred, it would seem that there was ample opportunity for leaf-curl infection to take place before the spray was applied on March 18. Standard 32° lime sulphur diluted 1–40 was used, since it was considered sufficiently effective to obtain control if infection had not already taken place. A stronger solution might have caused serious injury to the buds.

The 1935 experiment gave a striking demonstration of leaf-curl control, the sprayed trees showing an average of only 130 infected leaf clusters per tree, whereas there were 2,825 diseased clusters on the check tree.

EXPERIMENT IN 1936

A further check on the possibility of controlling leaf curl by spraying after the buds had swollen conspicuously or had made considerable growth, was sought in 1936. Spray applications were planned to begin while the buds were yet dormant and to continue at frequent intervals until the immediate pre-blossom stage.

A row of Elberta trees were selected in the Schmidt orchard near Winchester, an orchard that had been severely infested with leaf curl for several seasons. The trees varied in size and from 1 to 3 trees per plot, with most plots containing only 2 trees. All plots, with the exception of the check, were sprayed with lime sulphur 1–80. A knapsack sprayer was used, because soil conditions made impossible the use of a power rig (Table 2).

The results in 1936 were less conclusive than those of other years, although all applications were clearly beneficial. The first two applications were made with 16° Baumé lime sulphur through mistake. The later applications were made with 32° lime sulphur diluted to give the same strength, or 1 part in 80, so that the treatments would be uniform.

TABLE 2.—*Control of peach leaf curl obtained from delayed applications of lime sulphur in 1936*

Plot No.	Date sprayed	Stage of bud development	Number of diseased leaf clusters
1	March 5	Dormant	16
2	March 23	Swollen, silver tip	7
3	March 27	Leaves out $\frac{1}{8}$ to $\frac{1}{4}$ inch No color in blossom buds	21
4	April 1	Leaves out $\frac{1}{4}$ to $\frac{1}{2}$ inch	28
5	April 4	Leaves out $\frac{1}{4}$ to $\frac{3}{4}$ inch Pink in blossom buds	34
6	April 8	Leaves out $\frac{1}{2}$ to $1\frac{1}{2}$ inches Some blossoms open[a]	31
7	Check, no spray		92

[a] The peaches were in full bloom on April 14.

Rainfall throughout the duration of the experiment in 1936 was frequent and prolonged enough to give excellent opportunity for infection. Rain fell on 9 days between March 5 and 23; on 3 days between March 23 and 27; on 1 day between March 27 and April 1; on 2 days between April 1 and 4; on 1 day between April 4 and 8. There was a total of 9.09 inches of precipitation from March 5 to April 8.

EXPERIMENTS IN 1937

Eleven fungicides in one or more dilutions were tested in 1937 to check further their effectiveness in the control of peach leaf curl; but, since there

was not a significant amount of disease on the nonsprayed checks, no counts were made. No curl was observed on the trees in any of the sprayed plots. Additional information, however, was obtained on the control of the disease resulting from spray applied after conspicuous bud development. These experiments were located in a different orchard.

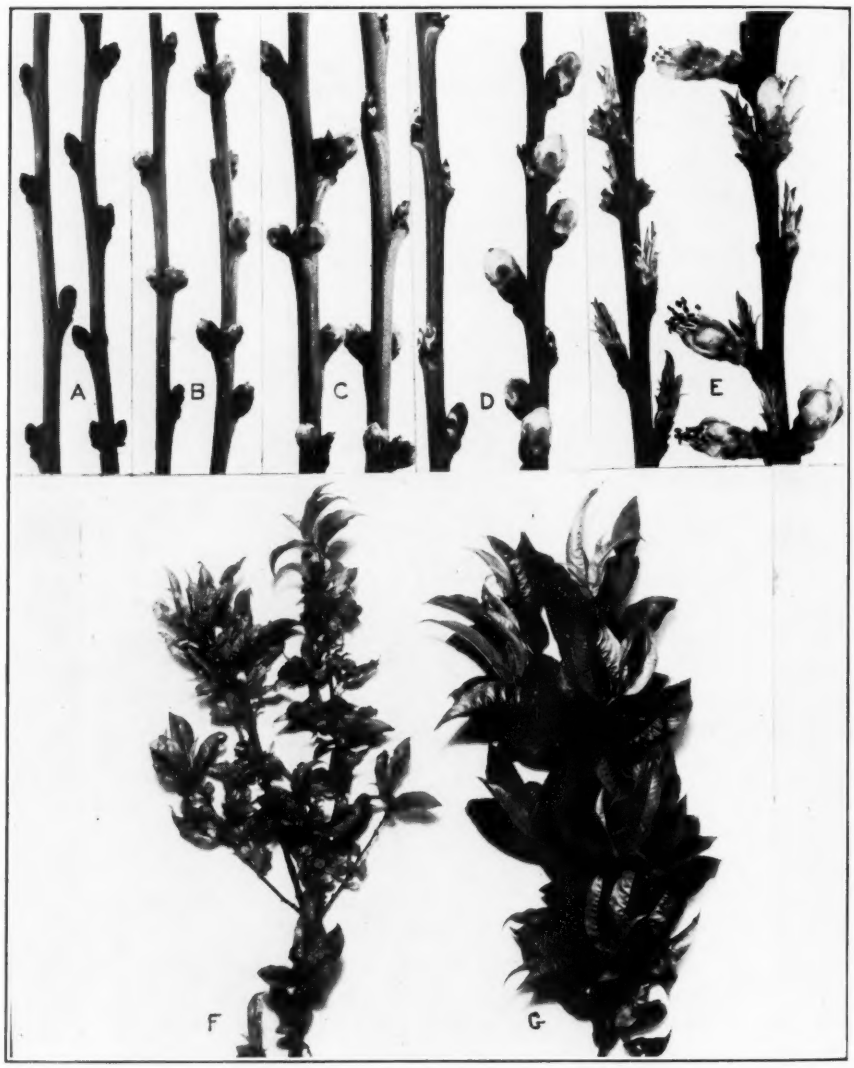

FIG. 1. Stage of peach bud development on dates of 1937-delayed-spray applications. A. Dormant application, March 3. B. Second application, March 9. C. Third application, March 12. D. Fourth application, March 23. E. Fifth and final application, April 1. F. Nonsprayed peach branches showing severe leaf-curl infection. G. Noninfected branch sprayed with 1–40 lime sulphur on March 23, 1937.

A number of old neglected trees in the Virginia Valley orchard near Stephens City, Virginia, were selected for the delayed fungicide applica-

tions. Each plot consisted of 2 trees, the checks being located between the sprayed plots. Lime sulphur 1–40 was applied with a knapsack sprayer, as previously. The first application was made while the buds were dormant, and successive applications were made at intervals as the buds developed. Shoots were cut off at the time of each spray and photographed (Fig. 1). The dates of the spray applications and the amount of leaf curl that subsequently developed are given in table 3.

TABLE 3.—*Peach leaf curl counts on Elberta trees sprayed at intervals from the dormant period until the first blossoms opened. Stephens City, Virginia, 1937*

Plot No.	Date sprayed	Number of diseased leaf clusters	Plot No.	Date sprayed	Number of diseased leaf clusters
5	April 1	149	1	March 3	72
6	Check, no spray	4590	2	March 9	18
7	Check, no spray	3370	3	March 12	30
			4	March 23	111

The degrees of control obtained with the delayed applications seem to indicate either that very little infection had taken place prior to April 1, or that the lime sulphur was able to kill or "burn out" the young infections. The rainfall for March was light, being only 1.15 inches. Two showers totalling 0.42 inch fell after March 15, the remainder of the precipitation falling earlier in the month in the form of snow.

DISCUSSIONS AND CONCLUSIONS

The ability of relatively weak fungicides or of dilute concentrations of strong fungicides to control peach leaf curl has been indicated from time to time in numerous scattered reports. That such observations are valid is supported by the results obtained in orchard experiments and reported in this paper. No difficulty was experienced in controlling the disease with Bordeaux mixture as weak as 2–4–100, with lime sulphur diluted 1–50 or with proprietary wettable sulphurs at 16 pounds per 100 gallons. Soluble sulphur (dry sodium polysulphides) gave excellent control at 8 pounds per 100 gallons of spray, the weakest strength tested. Lime sulphur in a 1–40 dilution was always effective in leaf curl control (Fig. 1, G) when used in spraying the buds in progressive stages of development. Lime sulphur 1–80 noticeably reduced the amount of leaf-curl infection in a limited trial, but was not completely effective.

There is no doubt that the strengths of fungicides generally recommended for control of peach leaf curl are effective. Lime sulphur at 1–8 will also control San José scale, but frequently its use at this concentration is not the most economical way to control leaf curl and San José scale. Lime sulphur and Bordeaux mixture at the concentrations normally used as summer sprays on apple will control peach leaf curl in Virginia, and there seems to be no valid reason for using them in stronger concentrations.

Either of these fungicides can be used in combination with oil emulsion to provide a combination fungicide-insecticide spray, except where soap has been used as the emulsifier for the oil. Bordeaux mixture may be used as an emulsifier where it is desired to prepare an emulsion in the spray tank, and it, together with the oil emulsion, provides the lowest cost combination spray.

Several investigators have obtained satisfactory control of leaf curl by spraying after the buds were conspicuously swollen or even after the young leaves had protruded. The results reported here substantiate these observations and, contrary to the common assumption, clearly indicate that the end of the period for the control of peach leaf curl is not necessarily coincident with the first active swelling of the buds. Good control was obtained repeatedly in these experiments with sprays applied after swelling and growth of the buds had begun and until the first blossoms were opening. No sprays were applied later than this stage of development. It is quite possible that seasonal conditions may occur in which control could not be obtained after evident bud activity, but this does not alter the fact that in several seasons, when leaf curl was generally prevalent, control was obtained with delayed spray applications. During these years, weather conditions during the period of bud development were favorable for infection.

The possible ability of liquid lime sulphur to burn out young leaf-curl infections was suggested by Fitzpatrick (5), and its ability to accomplish such action in the case of apple scab (*Venturia inaequalis*) infections as much as 72 hours old was demonstrated by Hamilton (6). There is a possibility that lime sulphur may act in the same manner against young leaf-curl infections when delayed applications are made. Some of the early investigators reported unsatisfactory results from sprays applied after bud activity had begun; and, as a matter of safety in general practice, it is no doubt best to apply the leaf-curl spray either in the fall or as early in the spring as possible, but at the same time it should be recognized that many growers cannot or will not complete the spray within the time limits normally set. Seasonal conditions and individual problems frequently delay applications beyond the time commonly advised. The results of other investigators, which have been reviewed here, together with the new data presented, indicate that where such a spray cannot be applied in accordance with the normal schedule, results from later applications are satisfactory frequently enough to justify completing a spray at any time prior to blossoming. A reasonably mild or weak fungicide should be used as a matter of safety at this time. Exception is not being taken to present recommendations, but it is suggested that recommendations for leaf-curl control should be revised so as to recognize the benefit that may be derived from delayed spray applications.

SUMMARY

Peach leaf curl was controlled with fungicides in much weaker concentrations than are usually recommended. Lime sulphur 1–50, Bordeaux mix-

ture 2–4–100, and wettable sulphurs at 16 pounds in 100 gallons have all given effective control.

Good control of leaf curl was obtained with sprays applied after the leaves were protruding as much as one inch from the buds. Lime sulphur 1–40 was used in the delayed applications.

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RELATION OF STOMATA TO INFECTION OF PINUS STROBUS BY CRONARTIUM RIBICOLA

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INTRODUCTION

While studying the environmental factors influencing infection of 5-needle pines by *Cronartium ribicola* Fischer, a detailed study was made of the stomata of needles of *Pinus strobus* L. because Clinton and McCormick (3) stated that the germ tubes from sporidia of *C. ribicola* enter the needles by way of the stomata. The presence of substomatal vesicles beneath the stomata in young needle infections led them to conclude that fungal invasion occurs through the stomata.[2] They failed, however, to demonstrate any direct connection between the substomatal vesicles and sporidia on the exterior of the needles. They attributed this failure to the fact that "not sufficiently young infections, those less than a week old, have been examined, and the exceedingly great difficulty in locating points of infection, since there is no external evidence to guide one within three or four weeks after this takes place."

An attempt was made to determine the nature and development of the substomatal vesicles and to gather various stomatal statistics that might possibly affect the entrance of germ tubes of sporidia of *Cronartium ribicola* through stomata. The results are presented in this paper.

METHODS

The structure and periodic movements of the stomata were studied at the Charles Lathrop Pack Demonstration Forest, Warrensburg, New York, during the summers of 1931 and 1932. The first summer was spent largely in developing a satisfactory method of measuring stomatal activity. The data secured in 1931 agreed with those of the following season, but the results recorded in this paper were obtained from June 20 to August 20, 1932. The studies concerned with the mode of needle penetration were done during the summer of 1933 at Warrensburg and the autumns of 1934 and 1935 at the New York State College of Forestry, Syracuse, New York.

The needle samples for the studies of stomatal opening and closing were taken from trees subjected to the several growing conditions subsequently described in this paper. It was deemed necessary to study the stomata in needles of young pines growing in pots, as well as those of older pines grow-

[1] The investigations here reported were made by the writer while employed as Agent in the Bureau of Plant Industry, United States Department of Agriculture, in cooperation with the New York State College of Forestry at Syracuse, New York. The stomatal measurements were made by Mr. J. L. Lowe under the direction of the writer. Mr. J. S. Kring prepared part of the materials used in the study of needle penetration.

[2] Drs. Clinton and McCormick most graciously permitted the writer to study their slides on several occasions and very kindly discussed their interpretations in detail.

ing under natural conditions, because the study here reported was only one phase of another investigation. The potted pines were 4 years old, having been grown in seed beds for 2 years, in transplant beds for 2 years, and planted in pots during April, 1932, after which they were placed in the open and subjected to the prevailing weather conditions.

In studying the opening and closing of stomata it was impossible to use an ordinary microscope and direct light, or a vertical illuminator, such as an Ultropaque, in order to view the stomata in living needles, because the stomatal pits[3] are filled with a substance which obscures the guard cells and stomatal pores. The porometer method (4; 6; 9, p. 328) of measuring stomatal opening did not lend itself readily to the work with pine needles. Hence it was necessary to work with preserved material and to choose a method whereby the guard cells were quickly killed and fixed in position and by which the obstructing substance in the stomatal pits was quickly removed. The alcohol-fixation method, suggested by Lloyd (7) and by Loftfield (8) and recently severely criticized by Hartsuijker (4) and by Oppenheimer (10), gave definite and satisfactory results with the needles of white pine, and was, therefore, used in this study.

Five needles each of 1930, 1931, and 1932 origin were removed from each of the selected trees at the beginning of every hour during 24-hour periods. A portion of the epidermis was immediately stripped from each needle and immersed in absolute alcohol. Within the hour the strips of epidermis were mounted in absolute alcohol and examined microscopically. After examining and comparing the stomata from the 5 different needles of each needle age, 25 stomata from a typical needle were measured and recorded for each of the needle ages. When the pores were 0.5 μ or more in width they were considered "open" and were measured with a calibrated ocular micrometer. Pore-openings less than 0.5 μ in width were approximated and are recorded in this paper as "partly open."

The data on stomatal structure were obtained largely from free-hand sections of living needles and, to some extent, from stained sections made into permanent mounts.

In order to study the penetration of the needles by the germ tubes of sporidia, living needles still intact on the trees were inoculated. Young potted pines were used, as well as naturally established trees. The needles were inoculated by suspending small sections of telium-bearing *Ribes nigrum* leaves above marked portions of pine needles. Only those infected *Ribes* leaves known to be producing sporidia at the time were used. When potted pines were used, they were placed in a damp chamber under conditions favoring the germination of sporidia. On trees growing in the open, the needles were inoculated in the early evening or during periods of cool, rainy weather. Sufficient needles were inoculated at one time to permit one or more samples to be taken every 6 hours, up to and including 72 hours. A few needles

[3] Throughout this paper the urn-shaped chamber formed by the accessory epidermal cells above and leading to the guard cells is designated as the stomatal pit.

were removed from trees, inoculated, and placed on wet paper in Petri dishes kept in a cooler at about 53° F. The samples were killed and fixed in chromo-acetic solution contained in appropriately labeled vials. Later, they were embedded in paraffin, sectioned, stained, and mounted.

ARRANGEMENT AND STRUCTURE OF STOMATA

The stomata of *Pinus strobus* are arranged in several rows extending longitudinally along the 2 flat sides (ventral surface) of each needle (Fig. 1).

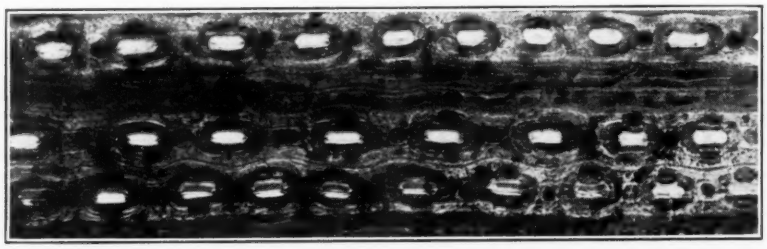

FIG. 1. Arrangement of stomata in needles of *Pinus strobus*. × about 120. (Photograph by the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.)

They are sunken below the epidermis and each communicates with the needle surface by means of an outer stomatal chamber or pit that, in mature needles, is plugged with a substance stated to be wax or a mixture of wax and resin (2, p. 214; 11; 12). In 1932, this material appeared in the stomatal pits above the sunken guard cells of the current-season needles about July 13, when the needles extended approximately 2.5 centimeters beyond the enclosing sheath and were somewhat over one-third the average length of the mature needles at the end of the summer period. As previously stated, it was these plugs that made it impossible to see the stomatal pores in living needles and thus necessitated the removal of strips of the epidermis and their immersion in absolute alcohol. The alcohol not only fixed the guard cells until the material could be examined, but it partially removed the waxy deposit and exposed the stomatal openings.

The first study was made in order to determine as nearly as possible the greatest distance from a stoma that a sporidium could be when it came to rest upon the ventral surface of a mature needle. This was considered to be the distance from the edge of a needle to a stomatal pore in the nearest row of stomata at the center of the long axis of the needle. The average distance between the pore openings of adjoining rows of stomata and the average distance from stomatal opening to stomatal opening of consecutive stomata within the same row also were determined. These distances were found to be approximately the same on needles taken from potted pines, from young pines growing in the open, and from older pines in the forest; therefore the data are combined in table 1.

The rows of stomata are unequally spaced on the needle surface, and varied from as little as 47 μ apart to as much as 201 μ. Within the rows,

TABLE 1.—*Stomatal statistics for needles of Pinus strobus*

Item	Needles of indicated origin					
	1930		1931		1932	
	Number measured	μ	Number measured	μ	Number measured	μ
Distance between edge of needle surface and pore of nearest row of stomata	75	165	75	161	75	141
Distance between pores of one row of stomata and the next	75	89	75	95	75	88
Distance between pores of consecutive stomata within a row	75	33	75	32	75	32
Depth of:						
Pit	62	15.2	75	14.7	69	14.5
Pore	62	10.8	75	11.2	69	10.9
Pit and pore	62	26.0	75	25.9	69	25.4

most of the stomata are so close to one another as to nearly touch, thus making the distance between the pores almost equal. The distance from the outer edge of the needle surface to the nearest pore varied but was rarely over 185 μ. In the 1930 and 1931 needles the distance was rarely less than 140 μ. The 1932 needles had apparently not attained their maximum width at the time they were examined, although they appeared to be mature. The maximum and minimum distances from the edge of the 1932 needles to the nearest pore were 192 μ and 102 μ, respectively.

In surface view each stoma appears as a pair of bean-shape cells with a depression between them (Fig. 2, A). These bean-shape cells are accessory epidermal cells, superimposed upon guard cells of similar shape (Fig. 2, B), the walls of which may be seen at the bottom of the depression or pit formed by the 2 accessory cells. The stomatal pore is considered as the opening between the 2 guard cells, and its depth the distance from the upper walls of the guard cells to an imaginary line drawn across the top of the substomatal chamber between and touching the bases of the 2 guard cells. In cross section the guard cells differ in appearance, depending on the location of the cut through the stoma (Fig. 2, D, E, F). Near the end of a stoma, beyond the edge of the pore, they have an additional wall thickness appearing as flange-like appendages (Fig. 2, F), so placed as to touch each other, and apparently are never drawn apart to enlarge the pore. At the edge of the pore (Fig. 2, E) they also appear to have flange-like appendages, but these are not observed in sections made at or near the center of the pore (Fig. 2, D).

Measurements of the depth of the stomatal pit and the pore were made, since this is the minimum distance a germ tube would have to grow in order to reach the interior of a needle. To this end needles were cross sectioned and only those stomata, sectioned at approximately the center of the pore, were measured. Again, it was discovered that the measurements were almost ex-

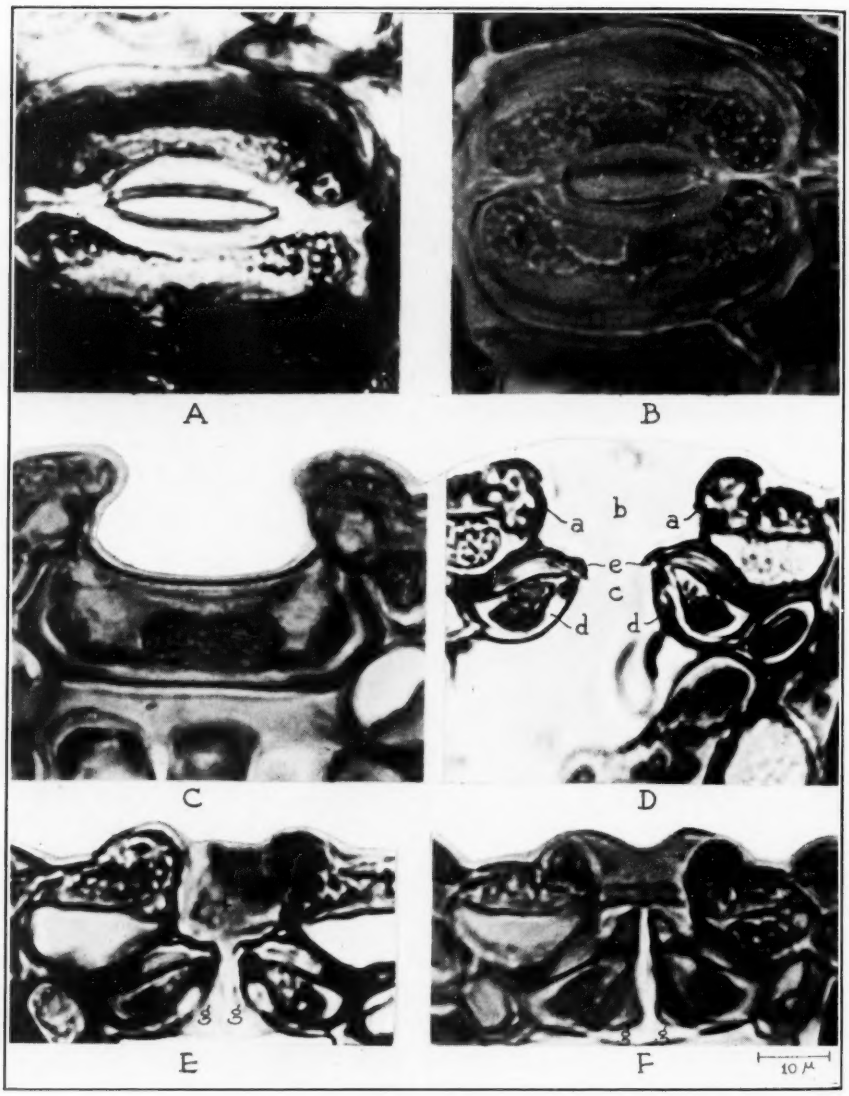

FIG. 2. Stomata of *Pinus strobus*. A. Surface view of a stoma showing 2 bean-shape epidermal cells above the guard cells. B. Surface view of the guard cells after the accessory cells have been removed. C. The side of a guard cell facing the pore. Note the large nucleus and the thick cell walls. D. Cross section of a stoma at center of pore: a, accessory epidermal cells; b, outer stomatal pit; c, pore (the processes involved in the preparation of permanent mounts affect the cells in such a way that the stomata are open wider than they actually occur in living needles); d, guard cells; e, beak-like projections of guard cells. E. Cross section of stoma near end of the pore showing a stomatal plug (f) and flange-like appendages (g) on the inner walls of the guard cells. F. Cross section of a stoma near the end of the guard cells away from the pore where the thickened walls remain pressed together.

actly the same for all the needles, regardless of their source; hence, the data are recorded in table 1 according to needle age only. There was relatively

little difference in the depth of the stomatal pits and pores in mature needles of like and different ages.

STOMATAL MOVEMENTS

The extreme edge of the walls of the guard cells at the pore appears in cross section as a beak with the upper or outer wall extending beyond the lower or inner wall (Fig. 2, D). The opening and closing of stomata of eastern white pine is in part the result of these beak-like extensions moving toward and away from each other. When a stoma is closed it is possible to observe in properly prepared mounts these extensions closely pressed together. In open stomata they seldom withdraw from each other more than 1.5 μ. Rarely are all the stomata of a needle open or shut at one time, although the majority may be.

As stated earlier in this paper, every hour during certain 24-hour periods, strips of epidermis from 5 living needles of each of 3 needle-ages were immersed in absolute alcohol, examined, and microscopically compared. In general, it was found that, for a particular needle age, the width of the stomatal pores on 1 needle were representative of the 5 needles. Twenty-five stomata from one of the 5 needles of each needle age were measured and recorded as the widths of the pores for a needle of a particular needle age for a particular hour.

At the time of maximum opening a few stomata opened as wide as 1.5 μ, very rarely to 3 μ; the majority of the pores averaged approximately 0.5 μ.

Studies of stomatal activity were made on pines of different ages, on different sites, and subjected to natural and artificial environments as follows:

1. A 12-year-old pine, growing in the open in sandy soil on the edge of a swamp.
2. A forest tree about 30 years old growing on an eastern slope in coarse gravel loam.
3. An 8-year-old pine, growing in sand in the open. Comparative studies were made on rainy days and on clear, relatively dry days.
4. A potted pine kept on the same site as the 30-year-old forest tree. Studies were made on these two trees during the same hours and days.
5. Three potted pines kept under damp-chamber conditions where the needles were continually moist. The temperature ranged between 50° and 60° F. and there was almost no light.

In addition, several forest trees approximately 50 years old growing on a rocky slope and several trees approximately 30 years old growing in a swamp were studied at various times during the summer of 1932 for the purpose of comparison with the more exact studies of the trees listed.

During the work in 1932, over 24,000 stomata were studied and equally as many in 1931 in preparation for the work of 1932.

Although there were some differences in the percentage of open stomata between the trees for specific hours, they were not significant. A large per-

centage of the stomata were open to some degree during every hour of the day, although the largest number was open or partly open between approximately 10 a.m. and 2 a.m. daily. This is shown in table 2, which represents

TABLE 2.—*Stomatal activity of a 12-year-old white pine from June 20 to July 22 and August 15 to August 20, 1932*[a]

Time		Condition of stomata					
		Current-season needles			Year-old needles		
		Closed	Partly open	Open	Closed	Partly open	Open
a.m.	*p.m.*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
8		51	34	15	64	34	2
10		40	34	26	56	36	8
12		35	38	27	51	38	11
	2	28	28	44	54	43	3
	4	26	28	46	48	41	11
	6	20	36	44	47	48	5
	8	25	28	47	42	53	5
	10	29	40	31	59	41	0[b]
	12	24	44	32	63	37	0
2		32	35	33	63	37	0
4		52	29	19	73	23	4
6		55	35	10	70	30	0

[a] Data based on 450 stomata on 18 needles per needle age for each hour indicated in the table.

[b] Although in the microscopic field all of the stomata may have been closed, there were always occasional stomata open on a needle.

the daily stomatal activity of a 12-year-old white pine between June 20 and August 20, 1932. The same stomatal activity occurred in the 8-year-old pine, both during periods of clear warm weather and cooler periods of rain. The stomata behaved the same on the forest trees as on potted pines subjected to natural meteorological conditions. In the damp chamber, however, nearly all the stomata on the potted pines were open to some degree between 8 a.m. and 8 p.m. and the majority were partly open during the remaining hours.

The stomata in the current-season needles were more active than those in the needles produced the previous year. The data for the needles of 1930 origin were taken but are not recorded in table 2, since they nearly parallel those for the stomata of the 1931 needles.

NEEDLE PENETRATION

Somewhat over 22,000 cross sections of inoculated needles were made into permanent mounts and studied for needle penetration by *Cronartium ribicola*. Approximately 2500 of the sections showed germinating sporidia present. In addition many temporary mounts were made and studied. The sporidia were in all stages of germination, some with germ tubes just appearing, others with tubes as long as 33 μ. Sporidia were found to have fallen not only near stomata, but many times directly upon the waxy plugs that filled the stomatal pits. None of the germ tubes were found to have grown through a stomatal pore into a needle; indeed, germ tubes of sporidia resting

directly above the stomata were observed growing along the epidermal surface away from the stomata. Commonly, the germ tubes from sporidia lying near stomata grew directly across them without any trace of downward growth toward the stomatal pores. This was true of germ tubes from both primary and secondary sporidia[4]: the latter being recognized by their placement near the ends of the sterigma-like germ tubes (5, fig. 4, p. 18) of the primary sporidia. In 7 instances, germ tubes growing from sporidia were found to penetrate the epidermal cells directly, some hyphae extending as deep as the hypodermal layer.[5] Penetration occurred through both the dorsal and ventral needle surfaces. The germ tubes entered the epidermal cells in 2 ways: 1. They retained their normal width as they passed through the cell walls. 2. They formed a fine, needle-like extension at their tip. No appressorium was present in either type of penetration, nor were any swellings of the germ tubes observed either within or outside the needle.

Although the writer failed to find substomatal vesicles associated with the phenomena of penetration, he observed vesicles present in established infections. Substomatal vesicles were present in living infected needles examined as long as 11 to 14½ months after the date of inoculation. Not only were substomatal vesicles present in the discolored spot, which is thought to denote the point of initial establishment of the fungus in a needle, but a substomatal vesicle was discovered in a section of a needle taken at a distance of somewhat over 3 mm. beyond the margin of the discolored spot and toward the base of the needle. This infection was 14½ months old; the mycelium had grown from the point of initial infection downward through the needle into the bark.

STOMATAL CHARACTERISTICS AS RELATED TO PINE INFECTION

It was not determined to what extent the waxy plugs in the outer stomatal chambers influence infection. These plugs appeared to consist of a mass of separate particles between which it seems possible that hyphae may force their way. The plugs normally filled the outer chambers but did not extend into the pores. Sporidia germinating upon living pine needles were examined by means of an Ultropaque. The germ tubes were found to be growing on the epidermis without any apparent regard for the stomata. The young hyphae were seen to grow between stomata as well as directly across and beyond them. Occasionally, hyphae were traced to stomata where they appeared to terminate, but it was impossible to see whether they grew downward into the pores. As previously stated, cross sections through such needles failed to disclose hyphae in the stomatal pit or pores.

[4] The production of secondary sporidia by primary sporidia of *Cronartium ribicola* appears to be a normal process and not the result of unnatural environment. Upon needlss of white pine they produce typical hyphae and as Spaulding and Gravatt (13) state they are no doubt "potentially dangerous to pines for much longer periods than the longevity of the primary sporidia would indicate."

[5] The examples of direct penetration have been examined by J. S. Boyce, G. G. Hahn, J. R. Hansbrough, and P. Spaulding of the Division of Forest Pathology, U. S. Dept. of Agriculture. The slides are now deposited in the Dept. of Forest Botany and Pathology at the New York State College of Forestry, Syracuse, N. Y.

For some time the writer has been studying the germination of sporidia of *Cronartium ribicola* placed on solid water agar and subjected to various temperatures (5). It was found that when freshly cast sporidia were kept at temperatures of 50° to 60° F. on solid water agar the following growth resulted at the end of the hours indicated in parentheses: No germination (2); young germ tubes on primary sporidia (4); secondary sporidia present and a few germinating (6); germ tubes of secondary sporidia averaging 10 to 11 μ (8); germ tubes mostly 20 to 40 μ (10); germ tubes mostly 20 to 50 μ (12 and 14); germ tubes mostly 20 to 60 μ (16); germ tubes mostly 40 to 100 μ (18 and 20); germ tubes mostly 40 to 106 μ (22). By means of an Ultropaque it was ascertained that, under similar conditions of moisture and temperature, the germ tubes from sporidia elongate at approximately the same rate on pine needles as on solid water agar. Table 1 shows that the average distance from the outer surface of a needle to the bottom of a stomatal pore is approximately 26 μ. If germ tubes enter a needle by way of the stomata, at least 10 hours must elapse following deposition of primary sporidia on the needles before the germ tubes can grow through the pit and pore, and then only provided the sporidia come to rest directly over the stoma. It has been observed (5) that at 50° to 60° F. primary sporidia tend to produce secondary sporidia; whereas, under the same conditions of temperature and humidity, secondary sporidia germinate and form true hyphae. Six hours after planting primary sporidia upon water agar they produced secondary sporidia that, 4 hours later, had germ tubes from 20 to 40 μ long. Therefore, if secondary sporidia are carried to a needle and fall upon the stomata, a germ tube may grow the distance from the needle surface to a substomatal chamber within a minimum of 4 hours. Hence, if the fungus invades the needles by way of the stomata, it is obvious that for needle infection to occur in a relatively short interval of favorable weather, sporidia must come to rest in the immediate vicinity of the stomata.

It is difficult to determine just what relation, if any, stomatal activity may have to needle penetration. A relatively large number of stomata were open to some degree at all times and would thus permit fungal invasion at any hour of the day. Most of the stomata were open between approximately 10 a.m. and 2 a.m. In the course of certain studies at Warrensburg, New York, in 1930, 1931, and 1932, a series of potted pines was exposed at stated intervals to natural infection by *Cronartium ribicola*. Equal numbers of pines were exposed daily from 8 a.m. to 8 p.m. and from 8 p.m. to 8 a.m.[6] One hundred and forty-two infections developed on 75 of the trees. Twenty-two of the infections were on trees subjected to inoculation between 8 a.m. and 8 p.m.; 120 infections occurred on those trees exposed between the hours of 8 p.m. and 8 a.m. Another series of trees was inoculated at the same time and in the same location as the first series but, following inoculation, the trees were placed for 36 hours in a damp chamber under conditions favorable to spore germination. In the second series, 151 infections developed upon

[6] These hours correspond approximately to periods of daylight and darkness.

77 trees; 4 infections occurring upon trees exposed between 8 a.m. and 8 p.m. and 147 on trees exposed between 8 p.m. and 8 a.m. Although several factors were involved that would definitely influence the amount of resulting infection, nevertheless, the fact remains that the greatest number of infections occurred on those pines subjected to inoculation when the fewest stomata were open. The fact that the number of open stomata and their size do not appear greatly to influence the amount of infection is demonstrated better in another experiment. During 1930, 1931, 1932, and 1933 a series of pines was subjected to natural inoculation by the blister-rust fungus. One hundred and ten trees became infected. At the time these 110 trees were subjected to inoculation, the year-old needles measured 173,260 linear inches; the current-season needles measured 157,310 linear inches. Through the year-old needles 152 infections occurred or 1 infection to approximately every 1100 linear inches of needles; through the current season needles 38 infections took place or 1 infection to approximately every 4100 linear inches of needles. In view of the fact that table 2 shows a greater percentage of stomata open in the current-season needles than in the year-old needles and that a greater percentage are open wider, it can be assumed that open stomata have relatively little effect upon the ability of the fungus to become established in the needles of white pine.

The fact that hyphae of *Cronartium ribicola* have been found to enter pine needles by direct penetration of the epidermal cells does not eliminate the possibility of entrance by way of the stomata. It is known that germ tubes from sporidia of rust fungi do penetrate their hosts in both ways but that direct penetration is more common than entrance through stomata (1). The fact that many sections with germinating sporidia upon them disclosed evidence only of direct penetration suggests that this may be the more common method with the blister-rust fungus.

The nature and significance of substomatal vesicles in needles of white pine are still to be determined. Arthur (1, p. 230), in referring to substomatal vesicles, states that "With the development of the hyphae the substomatal vesicle sometimes becomes divided by one or more septa, or it collapses and disappears, depending upon the species." In white pine needles substomatal vesicles are present in living needles a year or more after inoculation and are found even outside the discolored spot that is supposed to indicate the place of entrance of the germ tube and the establishment of infection. If substomatal vesicles in white pine needles are swellings of such delicate structures as young germ tubes, they would not be expected to persist for months after their formation, and if they mark the point of entrance they should be found only in the needle spots.

SUMMARY

The direct penetration of the epidermal cells by germ tubes from sporidia of the blister-rust fungus is reported for the first time.

It seems probable that stomatal activity has very little significance in the infection of *Pinus strobus* by *Cronartium ribicola*.

Stomata of *Pinus strobus* are arranged in rows on the ventral surface of the needles.

The average distance between the pores in contiguous rows of stomata was found to range from 88 to 95 μ; between the pores of consecutive stomata within a row, the average distance was 32 μ; and the distance in a straight line from any point on the margin of a stoma-bearing surface of a mature needle to the nearest pore averaged between 161 and 165 μ.

A stoma is opened and closed, in part, by the movement of the beak-like projections of the guard-cell walls.

Considering the depth of the stomatal pit and pore, it is probable that a minimum of 10 hours of favorable weather must elapse, following inoculation by primary sporidia of *Cronartium ribicola*, before a hypha can elongate sufficiently to come in contact with the mesophyll tissue by way of a stoma. In case of inoculation with secondary sporidia, the time may be shortened to 4 hours.

The greater percentage of the stomata were open between 10 a.m. and 2 a.m. This periodic opening occurred similarly in potted pines and pines growing under natural conditions, during periods of precipitation as well as intervals of clear weather with low relative humidity.

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INTERRELATION OF TAKE-ALL LESIONS ON THE CROWNS, CULMS, AND ROOTS OF WHEAT PLANTS[1]

HURLEY FELLOWS

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INTRODUCTION

When a wheat plant is attacked by take-all, the presence and relative severity of the disease on various parts of the plant are of interest, especially in estimating the degree of injury.

The present study was undertaken to determine the interrelations of the occurrence of take-all lesions, caused by *Ophiobolus graminis* Sacc., on the culms, crowns, and roots of wheat plants and the degree of severity of the disease on these structures.

METHOD

Data were obtained from wheat plants grown in soil that was naturally infested with *Ophiobolus graminis*. The plants were grown in pots or boxes in the greenhouse from 1928 to 1934, inclusive. Many conditions with respect to moisture, temperature, and the use of soil amendments and fertilizers and various chemicals not in the fertilizer class were represented. Results are not presented individually for each experiment, since, for the purpose at hand, they were substantially alike. The data were taken after heading but before maturity. The crown, culm, and roots of each plant were examined and placed in 1 of 5 categories according to severity of the lesions showing; viz., slight, slight-medium, medium, medium-bad, and bad. The number of diseased crowns, culms, and roots also was recorded, as were certain other data, such as height of plants, number of culms and plants, number of dead and living plants, number of plants that tillered, and number of heads formed. The data from each pot or box were recorded separately. Those from each pot or box are here designated a "test."

RESULTS

The data are presented in two different ways for clarity and completeness. One method consists of placing the plants in the 5 different groups, according to the percentage of diseased crowns. The average percentage of crowns, culms, and roots diseased; the average severity of the disease on the crown, culms, and roots; the percentage of roots lost; the percentage of plants dead; the average height; the percentage of culms heading; the percentage of plants tillering; and the total number of tests and plants involved for each group were determined (Table 1).

[1] Cooperative investigations by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Department of Botany, Kansas Agricultural Experiment Station. Contribution No. 354 from the Department of Botany, Kansas Agricultural Experiment Station.

TABLE 1.—*Average occurrence and severity of take-all on crowns, culms, and roots of five groups of Kanred wheat plants grown in the greenhouse under several conditions, the data being grouped according to percentage classes of diseased crowns*

Percentage classes of diseased crowns	Number of:		Average height of plants	Plants tillering	Culms heading	Plants dead
	Tests	Plants				
			Inches	*Per cent*	*Per cent*	*Per cent*
0.0	43	197	33.6	66.9	53.1	0.0
0.1 to 50	66	557	26.9	50.0	47.7	4.7
50.1 to 85	69	718	29.0	42.5	43.5	1.0
85.1 to 96	49	727	23.3	40.3	37.2	6.7
96.1 to 100	108	1808	19.8	22.3	24.2	14.3

Percentage classes of diseased crowns	Diseased:			Loss of roots	Average severity on:		
	Crowns	Culms	Roots		Crowns	Culms	Roots
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
0.0	0.0	0.0	2.4	1.9	0.0	0.0	10.9
0.1 to 50	36.7	10.7	26.7	12.3	23.3	15.3	37.3
50.1 to 85	63.6	13.0	44.5	27.1	33.8	17.1	43.4
85.1 to 96	89.7	25.0	69.7	31.0	49.5	24.3	54.3
96.1 to 100	99.4	26.6	65.4	42.1	61.9	30.2	66.7

The severity of lesions is expressed as the percentage of the greatest severity possible. The accuracy of this depends on the experience and judgment of the observer. In general it is based on the extent of the lesion and the intensity of the color. A comparison of the roots on a healthy plant with those on a diseased one is the basis for estimating the percentage of roots lost.

The other method of summarizing the data gives either (1) a comparison of the relative severity of the take-all on the crowns, culms, and roots, one group with another when all are diseased (Fig. 1), or (2) records occurrence only when one or two of the above organs or groups of organs are diseased and the third is not diseased (Fig. 2).

DISCUSSION

All the data indicate there is a definite interrelation between both the occurrence and severity of take-all lesions on the crown, culm, and roots. If the crown was not diseased, in only a relatively few cases were the culm and roots diseased. However, there were not many cases in which the roots alone were diseased, indicating that crown infection follows very soon after root infection. During the 5 years' study, only 4 plants were found in which the crown alone was diseased. In these particular instances there may have been diseased roots that were lost in handling.

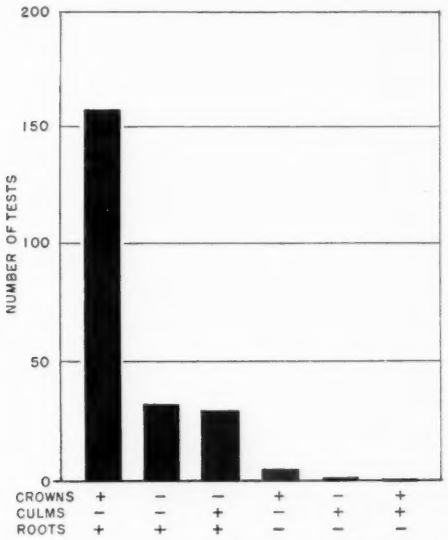

FIG. 1. Coincidence of presence and absence of take-all lesions on crowns, culms, and roots of Kanred wheat plants grown in the greenhouse in naturally infested soil. + indicates presence of lesions; −, absence of lesions.

An increase in the number of plants having diseased crowns is accompanied by an increase in the number of other diseased organs as well as an increase in the severity of disease on all parts. There is a slight exception

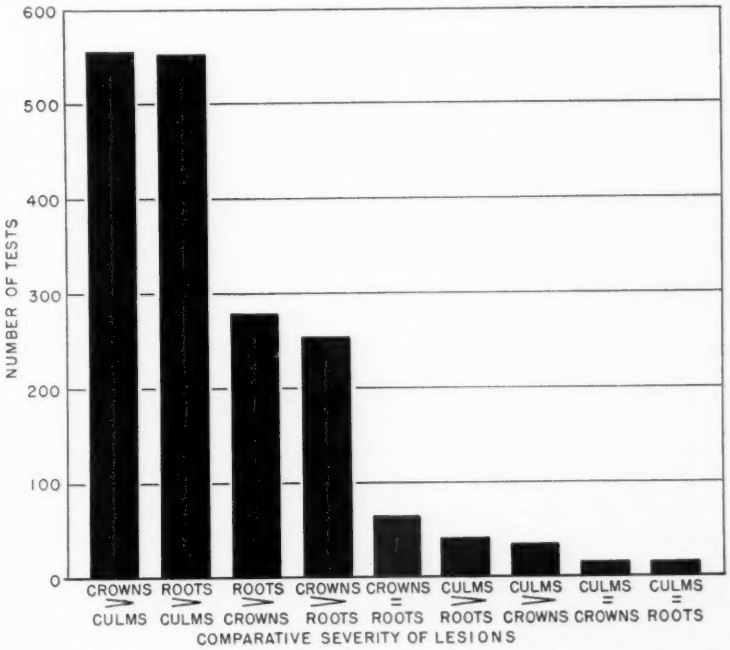

FIG. 2. Interrelations of severity of take-all on pairs of such organs as crowns, culms, and roots of Kanred wheat plants when all three of these organs were diseased. The symbol > indicates "greater than"; =, equal.

in the case of the percentage of roots diseased. This may be explained by the fact that new roots are formed during the life of the plant. Some of these roots may not have had time to become infected, and if infected, the time may have been too short for maximum severity. The loss of roots and the percentage of dead plants also increase with an increasing number of diseased crowns. The average height, the percentage of culms heading, and the percentage of plants tillering decrease, in general, with an increase in the number of crowns diseased. There is one slight exception in height, as shown in table 1.

As shown in table 1, as well as in figure 2, when only one of the 3 groups of organs is affected, it is usually the roots, indicating that they are the first to become diseased. Only once in 5 years has the culm alone been diseased and only 4 times were the crowns alone found to be infected. It is possible in these instances that diseased roots had been present but had been broken off in handling.

When 2 groups of organs were diseased and the third was not, it was found in the great majority of tests that it was the crowns and roots that were diseased and the culms not. The combination of culms and crowns diseased, and the roots free of lesions, has never been found. There were 29 of a total of 157 tests in which the culms and roots were infected and the crowns not (Fig. 1).

A comparison of the severity of lesions on the 3 organs under consideration shows, in general, the lesions of the crown and roots to be the most severe. Instances where the crown severity is greater than the root, and that of the root greater than that of the crown are about equal (Fig. 2).

There were 391 pots in which dead plants were found. In 387 of these, the crown, culm, and roots were diseased. Evidently, a wheat plant must be thoroughly invaded with *Ophiobolus graminis* before death occurs.

Sporulation was observed in 147 tests involving 1522 plants. In every instance where sporulation was noticed, all of the 3 organs under consideration were diseased and 29.7 per cent of the plants were dead. Sporulation was recorded only when it was clearly obvious. Evidently, abundant sporulation is associated with a high percentage of severity of the disease.

The casual observer depends on the black culm lesion for a diagnosis of take-all. If the disease has advanced enough for the typical culm lesions to show, one can be sure that in nearly all cases the crown and roots are diseased, and rather severely diseased.

SUMMARY

Observations were made on the interrelation of different degrees of severity of lesions on the roots, crowns, and culms of wheat plants attacked by *Ophiobolus graminis*. The data presented summarize 5 years' greenhouse work.

A definite interrelation was found between the presence and severity of lesions on the organs under consideration. If one organ alone was diseased,

it was usually the roots. Root infection was followed quickly by crown infection.

Increases in the percentages of crown infection were accompanied (1) by increases in root and culm infections; in the severity of lesions on all parts; in the loss of roots; and in the percentage of dead plants, and (2) by decreases in the average height of plants; in the percentage of culms heading; and in the percentage of plants tillering. All plants killed by *Ophiobolus graminis* and those on which sporulation was observed were found to have roots, crowns, and culms infected.

HISTOLOGY OF PHYMATOTRICHUM ROOT ROT OF FIELD-GROWN COTTON[1]

G. M. WATKINS

(Accepted for publication Nov. 30, 1937)

The mode of entry of the cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar, into the root tissues of susceptible plants has been studied by Peltier and Samson,[2] who reported that the fungus may enter the roots of field-grown cotton and alfalfa through such openings in the periderm as lenticels and wounds, and through ruptures caused by the emergence of lateral roots, or it may actually penetrate the intact periderm by mechanical action. According to them, the large central hypha of the superficial plectenchymatous strand proliferates in the direction of the underlying host cells. The branch hyphae thus formed grow through the external hyphal layers of the strand and produce a mycelial mass on the outside of the periderm. This mass is reported to assume a wedge-like form and to insert itself by mechanical force between the layers of cork cells. More recently, Peltier,[3] in discussing the relation between the strands and infection, has stated that "they are the sole agents responsible for the direct penetration and infection of the roots of susceptible plants." Butler,[4] working with watermelon plants infected with *P. omnivorum*, both in the field and under pure culture conditions *in vitro*, has reported in confirmation of Peltier and Samson that single or grouped hyphae invade the host tissue by a process of mechanical wedging.

In another paper[5] the writer has reported the results of a cytological study of cotton seedling roots infected *in vitro* under pure-culture conditions. This

[1] Published with the approval of the Director as contribution No. 424, Technical Series, of the Texas Agricultural Experiment Station. The progress of this study was greatly facilitated by a grant from the Texas Academy of Science for the purchase of materials and equipment.

[2] Peltier, G. L., C. J. King, and R. W. Samson. Ozonium root rot. U. S. Dept. Agr. Bull. 1417. 1926. (See Part II. The pathological anatomy of Ozonium root rot.)

[3] Peltier, G. L. Distribution and prevalence of Ozonium root rot in the Shelter-belt Zone of Texas. Phytopath. 27: 145–158. 1937.

[4] Butler, K. D. The cotton root rot fungus, *Phymatotrichum omnivorum*, parasitic on the watermelon, *Citrullus vulgaris*. Phytopath. 25: 559–577. 1935.

[5] Watkins, G. M. Cytology of Phymatotrichum root rot of cotton seedlings grown in pure culture. Amer. Jour. Bot. 25: 118–124. 1938.

study showed that infection is caused by newly formed hyphae that grow out from the inoculum, and that the typical buff-colored hyphae and strands do not appear until the attack is well advanced. Sections of the infected seedling roots usually showed degenerative changes in the nuclei and cytoplasm, abnormal shrinkage of protoplasts, and alteration in thickness and staining reaction of walls of cells several layers distant from the nearest invading hyphae. These abnormalities, together with the frequent occurrence of fragments of host cell walls in the midst of hyphal masses in more advanced infections, were considered as indirect but strong evidence of the liberation by growing hyphal tips of chemical substances, perhaps in the nature of enzymes, which facilitate disintegration of root cells in advance of penetration. Since the evidence appeared so convincing in favor of the power of the fungus to break down parenchymatous tissue by means of exudates absorbed in advance of hyphal entry, it has seemed well worth while to reconsider the problem of the histology of root rot of field-grown cotton and to investigate the possibility of similar processes being involved in fungous invasion of the suberized cells, which constitute the outer protective periderm.

The literature relevant to the general problem of host-parasite relationships in fungous diseases of plants has been well discussed by Brown,[6] and mention has been made previously[7] of a number of papers which seem to concern these relationships in cotton root rot.

MATERIALS AND METHODS

The cotton roots used in this study were collected from July to October of 1936 and 1937 from 3 fields in central Texas known to have been planted in cotton for several years and to have exhibited regularly, for a number of seasons, severe losses from typical Phymatotrichum root rot. Two of these places, the experimental root-rot plots of the Texas Agricultural Experiment Station and a commercial cotton farm 3 miles west of Bryan, are in Brazos County. The third is a cotton farm in Travis County. In collecting material for microscopic examination the plants were removed from the soil and the roots inspected immediately for the occurrence of typical hyphae or strands of *P. omnivorum*. Characteristically infected roots were obtained in all stages, from apparently normal roots bearing traces of delicate white hyphae to thoroughly rotted specimens completely surrounded by a buff-colored weft of strands and hyphae. All roots were placed in humid containers, brought directly to the laboratory, and sectioned at once by means of a sliding microtome. The sections resulting from this procedure ranged from 15 μ to 25 μ in thickness and were transferred as fast as cut to a preserving solution. Some of the sections were placed immediately in lactophenol for 15 minutes or more, transferred to another vessel of the same solution to which had been added cotton blue (0.5 gm. per 100 cc.) for 5 to 15 minutes, then

[6] Brown, W. The physiology of host-parasite relations. Bot. Rev. 2: 236–281. 1936.

[7] See footnote 5.

washed by passing through clear lactophenol, and finally mounted on slides in the same solution. Another lot was placed in Flemming's weak solution for several hours, washed in water, bleached in hydrogen peroxide, stained with safranin and light green, and mounted in balsam. The sections stained with cotton blue in lactophenol were better for studying the successive stages in hyphal invasion of the periderm, phloem, and cambium, while the permanent mounts stained with safranin and light green were superior for demonstrating hyphae in the xylem.

The examination of preparations obtained by the above methods has yielded information on the successive stages in the process of hyphal invasion of roots of normal field-grown cotton plants. Typical examples of the most significant stages have been drawn with the aid of a camera lucida.

OBSERVATIONS

The periderm of normal disease-free cotton roots of sufficient size to show advanced development of secondary tissues consists of an outer covering of cork cells, ordinarily 10 or 12 layers thick, with the cork cambium beneath and the phelloderm of parenchyma-like cells produced by the cork cambium on its inner side. As successive layers of cork cells are produced, the outermost layers of this tissue are constantly being sloughed off and lost. From the exterior of the intact cotton root this is evidenced by the appearance of minute cracks in the surface and the rolling back of extremely thin, scale-like layers of cells on both sides away from the original line of breaking. Frequently in roots in the earliest stages of mycelial attack the small white, more or less fluffy wefts are observed growing in the superficial crevices of the outer cork layers.

In transverse sections the ruptures in the surface of the cork tissue are seen to involve usually from 1 to 5 layers of cells, and it is in these crevices that the first advancing hyphae usually become established. At first the hyphae are loosely interwoven, but further growth generally transforms them into a more or less compact cellular mass (Fig. 1, a). As the separated layers of cork cells roll back away from the original rupture, the fungus is able to expand laterally from the original trench that it occupied and not infrequently is observed to extend the length of several cells down into the pocket formed beneath the separating roll of cork cells. The hyphae grow closely against the exposed walls of the cork cells (Fig. 1, a), but have not been observed by the writer to crush or give any indication of having exerted pressure upon the host cell walls.

Perhaps the most frequently observed phenomenon in this material is the change that occurs in the appearance and structure of those cork cell walls against which are lying hyphae of *P. omnivorum* (Fig. 1, a, b, c, d, f). Normally, almost hyaline in unstained mounts, these walls become brown, swollen, and sometimes distorted when the mycelium has grown in close proximity to them for a time. Occasionally, the walls appear under these conditions to be separated into laminae, although perhaps not to the extent frequently ob-

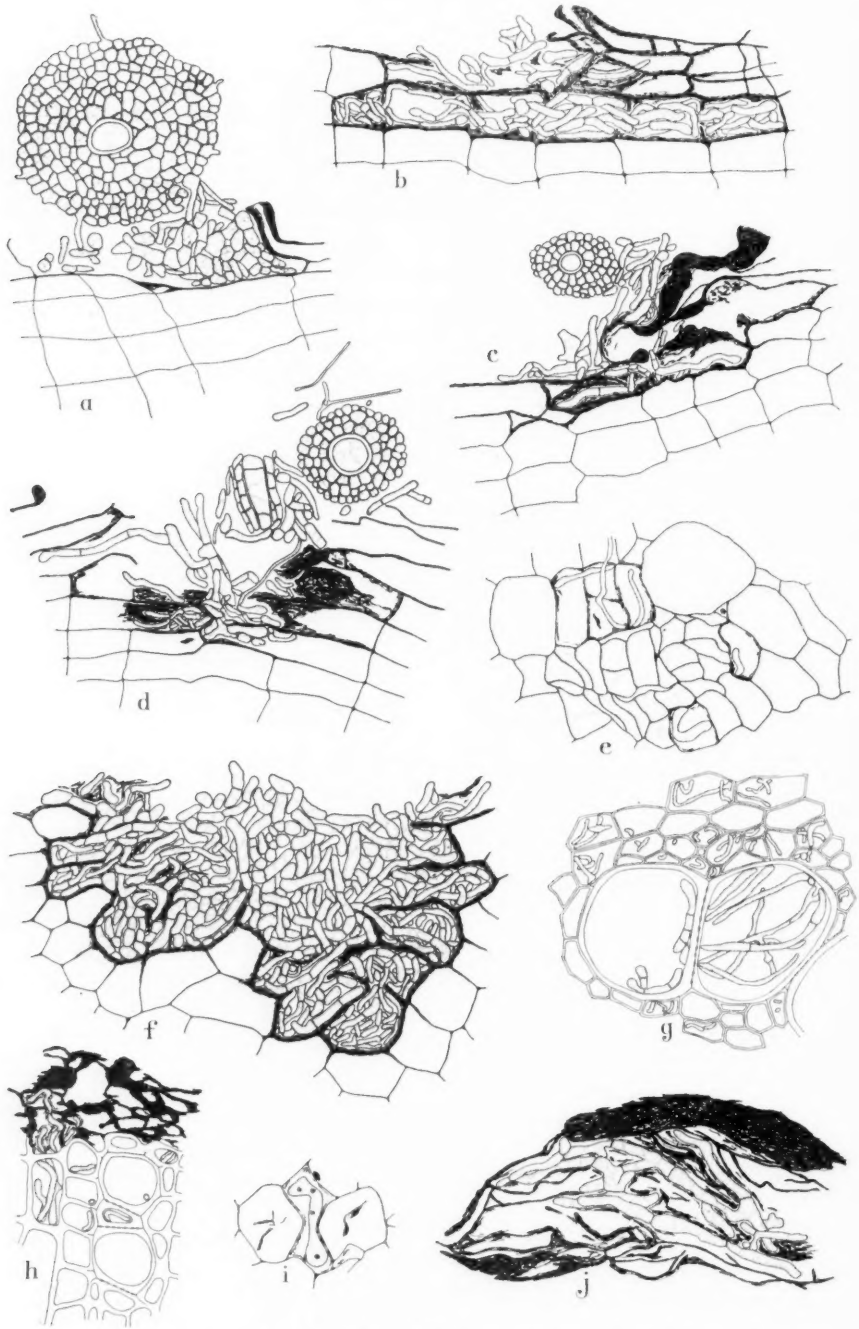

FIG. 1. a. Cross section of typical strand on surface of periderm. A group of hyphae are growing into the pocket at right formed by a separating layer of cork cells. b. Hyphae in a layer of cork cells beneath the surface. All walls in contact with hyphae are swollen and some are distorted. c. Cross section of strand at surface of root. Hyphae breaking down outer cork cells. d. Sections (transverse and oblique) of strands.

served in walls of the cortical parenchyma in seedling roots.[8] After this apparent chemical action on the wall has continued long enough to bring about its structural decomposition, breaks may occur that expose the cavity of the next adjoining cell and one or more hyphae from the accumulated weft soon grow into it (Fig. 1, b, c, d). The process is then repeated and continues in the same manner in all directions from the point of original establishment of the fungus. The spread of the hyphae is often at first more rapid tangentially than radially to the interior. Inasmuch as the hyphae, after entering each newly exposed cell cavity, soon undergo further growth and branching to occupy the additional acquired space, the entire cavity of the lesion comes to be filled with a more or less compact mass of hyphae that resembles a pseudoparenchyma in structure (Fig. 1, b, f). Around the entire zone of contact between this mass and the cork cells of the periderm are seen the remains of cork cell walls engulfed by the advancing hyphal mass but not yet completely broken down (Fig. 1, f). All intact host-cell walls in immediate contact with the hyphal mass show the brownish discoloration and increased thickness characteristic of the walls described above in similar juxtaposition to hyphae in the first stages of invasion. This process of progressive breaking down of successive layers of cork cells, followed promptly by invasion and consolidation of newly opened cell cavities, continues until the fungus has completely penetrated the cork covering of the root.

The further growth of the hyphae through the phellogen, phelloderm, and phloem is apparently a more rapid process. In these tissues products of the growing hyphae appear to be transferred readily to cells several layers distant, for the influence of some toxic substance can often be seen in the degeneration of cells before hyphal penetration occurs. The cells of the phelloderm and many of the older layers derived from the phloem are normally filled with starch grains, which begin to disappear as soon as hyphae enter these tissues. The hyphae proceed between and through the cells, and the invaded tissue soon disintegrates to such an extent that only darkened masses of partially broken-down cell walls and débris from degenerating protoplasts remain (Fig. 1, h, j). Here and there in the remnants of phloem tissue the network of hyphae can be seen (Fig. 1, j). The cells of the cambium and immediately adjacent phloem regions are among the first to collapse after the mycelium has penetrated within the periderm (Fig. 1, h), a condition that makes the observation of hyphae in close connection with cambium cells an infrequent occurrence (Fig. 1, e, i). In the phloem region the numerous

[8] See footnote 5.

Hyphae penetrating into cork cells beneath the surface and causing disintegration of walls. e. Hyphae in cells of cambium (oriented vertically in this illustration) and phloem. f. Hyphal mass that has penetrated about halfway through the periderm. Remains of cork walls are shown among the hyphae around the margin of the lesion. g. Hyphae in vessels, tracheids, and wood parenchyma. Two examples are shown of the passage of hyphae through pits. h. Hyphae in xylem cells and in the disorganized remains of cambium and phloem. i. A hypha between two cells of the phloem. j. Hyphal network in the disorganized phloem tissue. All illustrations ×175, except e and i, which are ×431.

bundles of bast fibers are especially conspicuous after the breakdown of the surrounding cellulose-walled cells. The fibers appear to be extremely resistant to the action of the fungus; and, although in advanced infections hyphae may occasionally be encountered within the lumina of fibers, no case of actual disintegration of bundles of bast fibers has been observed.

Sections of roots in advanced stages of decay usually show hyphae in great abundance in the xylem (Fig. 1, g, h), as noted by Peltier, King, and Samson.[9] At times cross sections of such material show hyphal distribution in more or less distinctly circumscribed areas in the wood, as though the principal direction of spread were lengthwise in the root rather than transverse. This type of hyphal distribution in the xylem may be considered as lending support to the conclusion of Peltier, King, and Samson that the mycelium travels readily in a longitudinal direction within the woody tissues. Radial growth seems to be accomplished more easily through the xylem rays, the cells of which are generally elongated in the radial direction. The slender hyphae so frequently found in the rays may travel more or less directly through several cells in tandem without being greatly diverted from the radial line of growth. The mode of wall penetration here is characteristically through the pits, as is true also of hyphae passing through walls of vessels, tracheids, and xylem parenchyma (Fig. 1, g). There is generally a considerable reduction in the diameter of the hypha at the point of passage. No evidence has been found of any chemical action upon the walls of xylem constituents; even those woody tissues permeated by a dense network of mycelium show no indication of decomposition of walls.

As far as has been observed in this study, the cotton root is unable to produce any defensive mechanism for inhibiting the fungus in its further spread after it has once traversed the periderm. The living cells of the phellogen, phelloderm, phloem, and cambium seem to be readily susceptible to damage considerably in advance of actual hyphal penetration. This, presumably, is brought about chemically by exudates from the active hyphal tips.

The observation of Peltier and Samson that the mycelium of *P. omnivorum* enters the root through lenticels and through ruptures in the periderm caused by the emergence of lateral roots is confirmed in the present work. Hyphae have been seen frequently to accumulate over the outer surfaces of lenticels and to grow through and between the loosely arranged cells beneath. In advanced stages of hyphal entry through lenticels the remnants of partly destroyed host cells are to be observed usually within the invading mass of hyphae, and around the borders of lenticels the mycelium often begins to grow into the exposed cells of the upturned phellem in much the manner described above for hyphal growth into cells beneath the edges of cork layers being sloughed off.

DISCUSSION

In its attack upon the uninjured, uninterrupted portions of the periderm of the cotton root, *Phymatotrichum omnivorum* shows much evidence in cyto-

[9] See footnote 2.

logical preparations of utilizing chemical exudates, probably enzymic in character, as a means of destroying the homogeneity of the matrix of the suberized walls. The process appears to be fundamentally similar to that described elsewhere[10] for the invasion of cotton seedling roots by the same fungus. The chief differences, readily discernible in a microscopic examination, are that, in the case of the attack on the cork cells of the periderm, the process of invasion proceeds much more slowly, and that the apparent changes in the composition and structure of the cork walls are not observed to have taken place farther in the host tissue than the walls immediately exposed to the action of the growing hyphae. In other words there is little apparent action in advance of actual hyphal penetration. This is possibly to be explained on the basis of the impermeability of the suberized walls of the outer periderm to the passage of solutions and the inability of fungous exudates to be absorbed and transferred beyond the intact wall next adjacent to the growing hyphae. The necessarily slow progress of the fungus through the resistant periderm accounts for the absence of the individual hyphal penetration through cell after cell so frequently seen in parenchymatous tissue. Since progress is slow and each cork wall is broken down only after being subjected to the action of the accumulated products of many hyphae, the fungus has opportunity for complete occupation of each cell cavity before the next one is opened.

In this work no examples have been encountered of the crushing of periderm cells adjacent to an advancing hyphal mass, and it thus seems unlikely that the penetration of the mycelium is due solely to mechanical force. On the other hand there is evidence strongly suggestive of the power of the actively growing hyphal tips to secrete and liberate some substance or substances that have the property of softening and dissolving suberized cell walls.

Present observations emphasize the dominant rôle in infection played by the actively growing portions of the mycelium. The fact that, in more than half the infection sites studied, the invading hyphae were not immediately associated with superficial plectenchymatous strands, tends to discount the necessarily direct relation between strand and invasion supposed by Peltier and Samson.

SUMMARY

In naturally occurring root rot of field-grown cotton the fungus, *Phymatotrichum omnivorum*, forms hyphal wefts that grow over the surface of the periderm and frequently accumulate in the superficial crevices resulting from the rupture and sloughing-off of the external cork layers. The periderm cell walls in immediate contact with hyphal agglomerations soon begin to show changes in structure, color, and thickness that suggest chemical action upon them by substances possibly liberated from the actively growing hyphae. Breaks occur in such walls, permitting the fungus to invade the newly

[10] See footnote 5.

opened cell cavity. As this process continues the number of invaded cells increases, and the fungus thus progresses slowly through the periderm. The center of the lesion is occupied by a compact hyphal mass that contains in its interior the engulfed remnants of partly destroyed cork walls. After having penetrated the phellem, the mycelium spreads rapidly through the phellogen, phelloderm, phloem, and cambium, causing widespread collapse and disintegration of cells, and enters the woody cylinder. The passage of hyphae from cell to cell of the xylem is chiefly through pits, since the lignified walls of these cells are not readily broken down by the fungus.

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PECTASE ACTIVITY OF CERTAIN MICROORGANISMS[1]

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(Accepted for publication Dec. 6, 1937)

Pectic enzymes are known to occur generally in tissues of higher plants and within cells or in secretions from certain microorganisms. In certain plant diseases characterized by the dissolution of cells in advance of the invading pathogen, the pectic cementing materials binding the structural elements of plant tissues are broken down, presumably by the pectic enzymes secreted by the pathogen. These enzymes include protopectinase, pectase, and pectinase, according to the nomenclature suggested by Davison and Willaman (3). Pectic activity of microorganisms having a relationship to parasitism has been determined by methods based upon the dissolution of cell walls (by protopectinase) of selected tissues (2), (6), and (8), the time of coagulation and consistency (by pectase) of pectic gels (3), and end products (by pectinase) from pectin (3). Certain disadvantages encountered with these methods and the importance of pectic enzymes in diseases of plants prompted the investigations on a method (10) for determining pectase activity based upon the hydrolysis of a known substrate. Pectase hydrolysis disrupts the methyl ester linkage in pectin with the production of methyl alcohol and pectic acid which, in the presence of calcium ions, goes to calcium pectate. According to Neuberg and Ostendorf (10) extracts from plant tissues possessing pectase activity hydrolyze the ester linkage of half calcium salt of monomethyl tartaric acid. Since the substrate is water-soluble and is hydrolyzed by pectase into soluble methyl alcohol and insoluble half calcium salt of tartaric acid, the method has promise of quantitative measurements based upon the precipitate formed. The purpose of this paper is to call attention to this method and to report the data obtained from determining pectase activity of certain plant pathogens, available in the laboratory, and an extract of cured-tobacco leaves by this method.

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station, and is published by permission of the Director.

[2] Now with U. S. D. A. Bureau of Plant Industry, Washington, D. C.

METHODS

The various organisms were cultured for 5 days at 37° C. in 100 mls. of nutrient broth containing 10 per cent commercial pectin, which was added to favor the production of pectase (7) and (8). At the end of the incubation period the cultures were adjusted to a reaction of pH 8.5 in 0.1 molar phosphate buffer and filtered through filter paper. The mycelial mass of the fungal pathogens in the residue and 25 grams of cured-tobacco leaves were macerated separately with mortar and pestle and then extracted in 100 mls. of 0.1 molar phosphate buffer at pH 8.5 for 6 hours at room temperature. The filtrates from these extractions and the filtrate from the culture fluids were added to an equal volume of 95 per cent ethyl alcohol for precipitation of the enzymes (1). The precipitated materials were collected on filter paper and, after being dried rapidly, were kept until needed in the experiments. The dried material was extracted in 2 mls. of 0.1 molar phosphate buffer at pH 8.5 and the clear liquid after passage through filter paper was used immediately as the preparation of pectase.

The ester substrate (half calcium salt of mono-methyl tartaric acid) was prepared[3] according to the method of Guérin-Varry (5), since the substance was not purchasable at the time. Five mls. of a 10 per cent aqueous solution of the crude syrup of the ester (containing also di-methyl ester) after being adjusted to a reaction of pH 6.5 in acetate buffer was added to the pectase preparations. Upon mixing the liquids and adding 0.5 ml. of toluol to prevent bacterial growth, the solutions were incubated at 37° C. for 1 to 5 days. A precipitate in the tubes indicated hydrolysis, which is considered to be pectase activity.

RESULTS

Qualitative data on the hydrolysis of the ester substrate by the preparations from cultures of the pathogens and from cured-tobacco leaves are given in table 1. They indicate hydrolytic activity from certain cultures and from tobacco tissues. The cultures of bacteria possessed but slight activity. Those of *Fusarium* that had been transferred several times on artificial media since isolation from diseased tobacco stems showed very slight or no activity, while freshly isolated cultures gave moderate hydrolysis. The cultures of *Sclerotium* gave good tests, while those of *Sclerotinia* yielded but a fair amount of precipitate. Two specimens of a culture of *Rhizoctonia* that had been isolated for some time from diseased tobacco stems gave negative tests. The cultures of *Thielaviopsis* yielded positive but varying results. Upon artificial inoculation[4] culture 538, which possessed moderate pectase activity, produced severe black-root rot of tobacco, while culture 535, with slight pectase activity, produced less severe rotting. Culture 536, with marked pectase activity, was not inoculated. Extracts from cured-tobacco leaves possessed a fair amount of activity.

[3] Aided by Dr. Charles Barkenbus, Department of Chemistry, University of Kentucky.
[4] Unpublished data furnished by Dr. E. M. Johnson.

TABLE 1.—*Qualitative determinations of pectase activity of various plant pathogens and cured-tobacco tissue*

Organism	Material	Results
Phytomonas mori (B. & L.) C. S. A. B.	Fluid	+[a] slight
P. tabaca (W. & F.) C. S. A. B.	"	+ "
P. angulata (F. & M.) C. S. A. B.	"	+ "
Bacteria from tobacco stem	"	+ "
Fusarium sp. from tobacco stem 484	"	++
F. " " " " 439	"	–[b]
F. " " " " 436	"	+ slight
F. " " " " 434	"	–
F. " " " " 477	"	+ slight
F. " " " " 302	"	+ "
F. " " " " 255	"	+ "
F. " " " " 227	"	+ "
F. " " " " (freshly isolated)	"	++
F. " " " " (all above combined)	Mycelia	+
Sclerotium bataticola Taub. 1	Fluid	+++
S. " 2	"	++
S. " (1 and 2)	Mycelia	++
Sclerotinia sclerotiorum (L.) Mass.	Fluid	+
S. trifoliorum Erik.	"	+
Rhizoctonia sp. from tobacco 540	"	–
Thielaviopsis basicola (B. & B.) Zopf 535	"	+ slight
T. " 538	"	++
T. " 536	"	+++
T. " (all above combined)	Mycelia	+++
Tobacco (tissue extract)	Fluid	+
Control (without enzyme)		–

[a] Amount of precipitate, positive test.
[b] Absence of precipitate, negative test.

DISCUSSION

The tartaric acid-ester method of measuring pectase hydrolysis appears to be adaptable for studies on plant pathogens, since positive results were obtained from certain organisms in culture. Because a known substrate is used this method may be more useful than those methods previously employed. Since plant tissues may contain inactive pectic enzymes capable of being activated by treatment or by definite substances, the tissue method might lead to erroneous results if the preparation tested for enzyme activity contained activators. Under such conditions enzymes present in the tissue would be measured instead of, or along with, that of the preparation. Davison and Willaman (3), by their method of preparing pectin, eliminated enzymes from the substrate, but their method of measuring enzyme activity requires considerable time and apparatus. The tartaric acid ester method (10) eliminates enzymes from the substrate and affords a simple and rapid means of determining pectase activity. It is adaptable for quantitative measurements.

Since the characteristic nucleus of the pectic materials appears to be digalacturonic acid (9), pectase presumedly acts on the ester linkage of the acid with methyl alcohol in the pectin complex. Some evidence (10) indicates that lipase from *Ricinus* seeds also hydrolyzes the methyl ester linkage in

pectin. Since pectic enzymes are generally present in plant tissues this lipase may have contained pectase.

Alteration of the physical and chemical properties of soluble pectin by the action of pectase may account for certain pathological expressions, although the rôle of pectase in the symptoms of plant diseases is not known. The substrate for other pectic enzymes (protopectinase and pectinase) is not definitely known, but the enzymes are apparently associated with certain pathogens. In plant diseases characterized by dissolution of the cells in advance of the pathogen, protopectinase (in some references pectinase) has been considered the causative agent. Its action changes the insoluble pectic material, cementing the structural elements of cell wall into soluble pectin. Since the action of an enzyme on an insoluble substrate is difficult to explain, the dissolution of cells may result from the action of pectase on soluble pectin that may be present in the cell walls. The pectic acid produced from such hydrolysis would be precipitated by the calcium ions in the tissue to insoluble calcium pectate (4), which has gelling properties. The forces of gelation of the hydrophyllic calcium pectate might disrupt the cell walls.

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A SAND–NUTRIENT INFECTION TECHNIQUE FOR THE STUDY OF FUSARIUM WILT OF COTTON

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(Accepted for publication Dec. 1, 1937)

Investigations of the nutritional relations of fusarium wilt of cotton at the Arkansas Agricultural Experiment Station have necessitated the development of a sand-nutrient infection technique. The method reported here was first used in modified form to determine the influence of variations in the nutrient supply on the relative incidence and severity of cotton wilt under greenhouse conditions. Through the information obtained from repeated trials it has been found effective to apply inoculum as a 3-day growth of the pathogen on modified Richard's solution. By growing the organism at 28–30° C. and keeping it well dispersed in the solution by shaking, it was found to produce a maximum of spores and active mycelium fragments with a minimum of staling products in the 3 days. This exclusion of all possible organic material and fungus staling products seemed necessary to the accurate determination of nutrient effects. The method described is relatively rapid, since preparation can be completed within a week and a significant disease differential obtained within 6 weeks after planting. These preliminary experiments showed such promise that the method was tested on a series of cotton varieties of known[1] relative wilt resistance under field conditions. The comparative resistance of these varieties was shown to be relatively the same, by this method of testing in the greenhouse, as has been demonstrated under very severe field conditions.

THE METHOD

Twenty-four 3-gallon glazed earthenware jars, with center drainage holes in the bottom, were equipped with siphon drains so that moisture would be automatically removed if the level of the liquid rose over 1½ inches from the bottom (Fig. 1). These jars were then filled to within two inches of the top with washed river sand that had been pasteurized[2] at 80° C. Three days prior to planting an actively pathogenic isolate of *Fusarium vasinfectum* Atk. was inoculated into 24 1-litre Erlenmeyer flasks, each containing 500 ccm. of sterilized Richard's solution made up in tap water with iron omitted. These flasks were shaken vigorously every 8 hours and incubated for the 72 hours at 28°–30° C. One flask of the inoculum was then mixed thoroughly

[1] Ware, J. O., V. H. Young, and G. Janssen. Cotton wilt studies. III. The behavior of certain cotton varieties grown on soil artificially infested with the cotton wilt organism. Arkansas Agr. Expt. Sta. Bull. 269. 1932.

[2] The ½-yd.-capacity pasteurizer used was made according to the directions supplied by the General Electric Company, from which the heating units were purchased. The use of this type of pasteurizer is described by J. G. Horsfall. Pasteurizing soil electrically to control damping-off. New York (Geneva) Agr. Expt. Sta. Bull. 651. 1935, and by Newhall, A. G., and M. W. Nixon. Disinfesting soils by electric pasteurization. New York (Cornell) Agr. Expt. Sta. Bull. 636. 1935.

with the sand in each of the 24 jars. An additional inch of moist, pasteurized river sand was spread over the surface of each jar, then 20 sulphuric acid-delinted seeds were planted in each jar in a circle 1 inch from the periphery and 1 inch deep. Four jars were planted to each of the 6 varieties: Half and Half (Summerour's), Misdel-3, Rowden 2088 (Arkansas), Dixie Triumph-6 (Arkansas), Rhyne's Cook, and Sea Island (Seabrook).

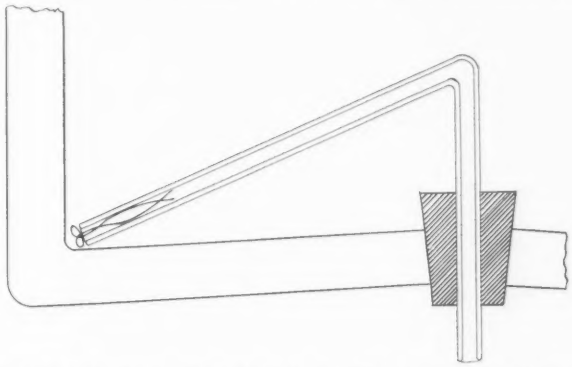

FIG. 1. Siphon drain for sand nutrient jars. The 3-mm. bore glass tube is shaped to have the bend 1½ inches above the bottom of the jar. A fine tinned wire is bent and placed in the upper end of the tube to prevent clogging with sand.

The nutrient solution was added at the rate[3] of 1 quart per jar per day, starting on the day following planting and continuing without flushing until the termination of the experiment. The nutrient formula used for this experiment is given in terms of milliliters of half molar solutions of salts to be made up to 10 quarts with tap water.

Calcium nitrate, 12.5 ml.; ammonium nitrate, 7.25 ml.; potassium acid phosphate, 7.25 ml.; magnesium phosphate (primary), 7.25 ml.; magnesium sulphate, 7.25 ml.; (all chemicals used were of C.P. quality).

The plants were thinned to 10 per jar on the 14th day and then the second inoculation, using inoculum prepared in the exact manner previously described, was applied at the same rate as before. This time, instead of mixing with the sand, it was poured into a circular depression made 1 inch inside the circle of plants by pressing an inverted Büchner funnel, 5 inches in diameter, into the sand to a depth of about 2 inches. The sand was again leveled. Inoculum was prepared in this same way and introduced into the jars in this same manner on the 21st day following planting and again on the 28th.

This experiment was conducted from July 26 to September 18, 1937. For the first 5 weeks of this period, during which time essentially all of the severe wilt occurred, the mean, daily air temperature of the greenhouse averaged 29.18° C. This temperature is rather close to the optimum reported for this disease under greenhouse conditions.[4] Preliminary experiments

[3] Rate and concentration of nutrients used in the preliminary experiments were suggested by Dr. Cecil H. Wadleigh, Assistant Agronomist, Arkansas Agricultural Experiment Station, Fayetteville, Arkansas.

[4] Young, V. H. Cotton wilt studies. I. The relation of soil temperature to the development of cotton wilt. Arkansas Agr. Expt. Sta. Bull. 226. 1928.

TABLE 1.—*Development of fusarium wilt in 6 varieties of cotton in sand-nutrient culture. Plants grown in 3-gallon jars in the greenhouse from July 26 to September 18, 1937. Average daily mean air temperature of the greenhouse for the first 35 days: 29.18° C.*

Variety	No. of jars	Total plants	Plants with advanced wilt symptoms by—			Final disease analysis (54 days)					
			28 days	35 days	42 days	Plants entirely dead	Plants dwarfed and badly blighted	Plants slightly blighted	Plants internally discolored but having no external symptoms	Plants healthy	Total having external symptoms
			Per cent	*Per cent*	*Per cent*						*Per cent*
Half and Half (Summerour's)	4	40	100	100	100	40					100
Misdel #3	4	40	90	100	100	36	1	3			100
Rowden 2088 (Arkansas)	4	40	72.5	80	80	28	3	7	2		95
Dixie Triumph #6 (Arkansas)	4	40	32.5	55	55	21	5	8	6		85
Rhyne's Cook	4	40	17.5	22.5	25	8	6	9	17		57.5
Sea Island (Seabrook)	4	40	0	0	0				29	11	0

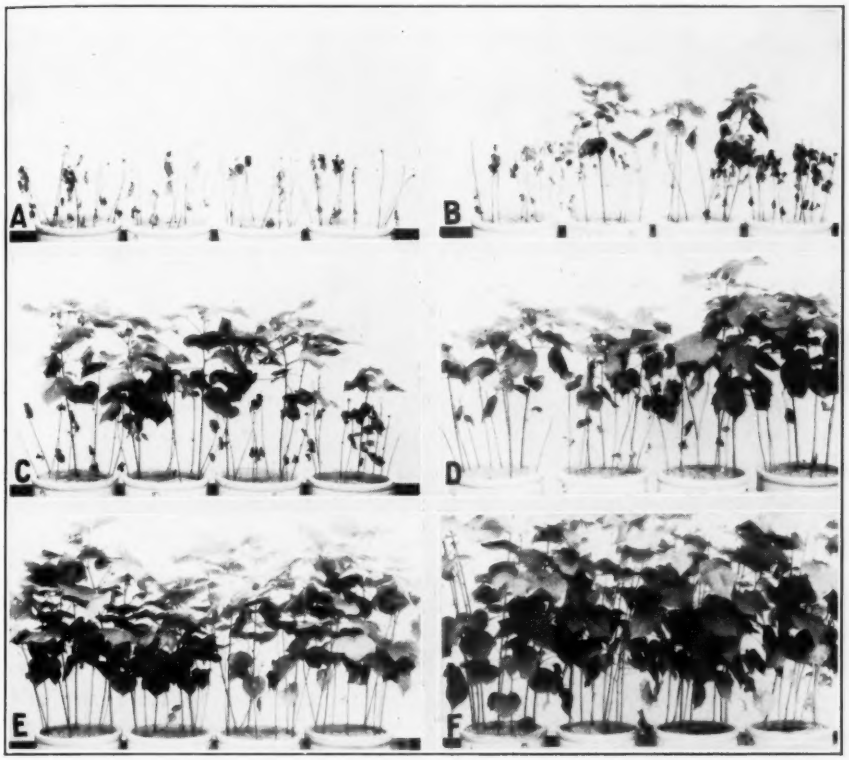

FIG. 2. Fusarium wilt in 6 varieties of cotton grown for 49 days in artificially infested sand-nutrient cultures. A. Half and Half (Summerour's). B. Misdel #3. C. Rowden 2088 (Arkansas). D. Dixie Triumph #6 (Arkansas). E. Rhyne's Cook. F. Sea Island (Seabrook).

with this method demonstrated that when temperatures are very much higher or very much lower than the optimum a high incidence of disease failed to develop. The data obtained from this experiment are shown in table 1 and the diseased condition of the different varieties, after 49 days, is shown in figure 2.

The first wilt in any variety (Rowden 2088) was recorded on the 21st day and within 1 week of this time the entire 40 plants of the most susceptible variety (Half and Half) were all showing advanced wilt symptoms. It was thus necessary to take notes at least once each week, for, quite obviously, *time* becomes one of the elements in measuring resistance when such rapid development and such high incidence of disease are induced.

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INOCULATIONS WITH FOREST TREE RUSTS

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In June, 1927, near Woodgate, N. Y., several species of pine and of the Scrophulariaceae were inoculated with the aeciospores of the *Peridermium* known as the Woodgate rust. The inoculations were kept moist by the use of a celluloid "iceless refrigerator," which is a modified form of the inoculation chamber described by Hubert.[1]

The 1927 terminal leaders, side shoots, and side branches of 5- to 20-year-old pines were moistened thoroughly and were smeared with aeciospores by means of a small artist's brush. A celluloid cone, which was 22 inches in length and respectively 3 inches and 1–1½ inches in diameter at the ends, was slipped over the inoculated parts. It was anchored to the stem by means of a copper wire. A strip of absorbent cotton, about 1 inch in width and 8–10 inches in length, which was saturated with water, was then pushed by means of a small rod through the smaller end, well down the inside of the cone. The entire outer surface of the cone was wrapped to a thickness of 1–1½ inches with wet absorbent cotton. The larger end of the cone was left almost entirely open, the smaller almost entirely closed, leaving an opening sufficiently large for circulation of air through the cone. If the cone was on the terminal shoot of the main axis of the tree, it was supported by one or two sticks, wired to the trunk of the tree; if on a side branch, it was supported from the ground by means of a forked stick (Fig. 1). The cones were left on the shoots for 24 to 36 hours. At the end of 36 hours the cotton on the outside of the cone was quite damp and it was always possible to wring water from the strip of cotton inside the cone. The entire outer surfaces of the shoots were also quite damp.

About 200 shoots of *Pinus resinosa*, *P. sylvestris*, *P. radiata*, and *P. ponderosa* were inoculated by this method. In all cases, infection spots appeared on the stems within four to six weeks, and in many cases, on *Pinus sylvestris*, the spots were so abundant that the entire inoculated stem surface became reddish brown in color by the latter part of August. This was equally true of trees which had no galls on them at the time the inoculations were made and of those which had galls.

At the time the shoots were inoculated, comparative germination tests of aeciospores from collections of various ages were made in the inoculation chambers and in water cultures in the laboratory. The water cultures were made by dusting the spores on fresh well-water in wine glasses. In the inoculation chambers pieces of thoroughly clean, white China silk about 2 cm. in length and 1 cm. in width were laid on the pine stems and the aeciospores were dusted on them. The silks were examined under the microscope 24 to 36 hours later. The germ tubes were very much branched and

[1] HUBERT, ERNEST E. Celluloid cylinders for inoculation chambers. Phytopath. 6: 447–450. 1916.

anastomosed so much with one another that they formed very complicated mycelia. In the water cultures, the germ tubes of the same age were comparatively unbranched and unanastomosed. In all of these experiments the percentage of germination in the inoculation chambers was far greater than that in the water cultures. For example, the germination of spores which had been kept in Syracuse watch glasses in the laboratory away from direct sunlight for about 10 days was approximately 25 per cent as compared with 75 per cent in the inoculation chambers.

FIG. 1. Celluloid "iceless refrigerator" used in inoculation experiments with the aecio spores of the Woodgate rust on *Pinus sylvestris* and other species of hard pines.

It is possible that Woodgate rust may prove to be one of the western gall rusts, whose alternate hosts are species of the Scrophulariaceae. Although several species of Scrophulariaceae occur in the region where the Woodgate rust has been found, none of them has ever been found infected with any rust. During the latter part of June, 1927, *Chelone glabra* and *Scrophularia leporella* were inoculated with aeciospores of the Woodgate rust. These two species were selected because of their size and because they occur nearby.

The plants when inoculated were about 26 inches in height. Both sides of the leaves were thoroughly moistened and smeared with aeciospores as mentioned above for pines. A stake about $\frac{3}{4}$ inch in diameter, wrapped with strips of wet absorbent cotton to a thickness of $1\frac{1}{2}$ inches was thrust into the ground beside the plants, so that its upper end extended 4 inches

above them. A celluloid cylinder, 22 inches long and 5 inches in diameter, was placed over the plants and stake, and rested on the ground. It was wrapped with wet absorbent cotton, which extended almost to the tip of the stake so as to leave the end of the cylinder partly open. After 48 hours, the cotton on the stake was wet and the leaves were quite moist. Microscopic examinations were made of pieces of the inoculated leaves soon after the cylinders were removed. The aeciospores had germinated as abundantly and the germ tubes were as profusely branched and had fused as freely as described above. No infection resulted in any of these experiments.

The writer and his assistants have made several thousand field inoculations with aciospores, urediniospores, and sporidia of *Cronartium ribicola*, using the celluloid cones, the celluloid cylinders previously mentioned, and the iceless refrigerators of the type described by Hunt[2] and Snell.[3] The results with the celluloid cylinders were almost entirely negative; those with the "Hunt iceless refrigerator" were fairly satisfactory, though less so than anticipated. Considerable labor is involved in using the "Hunt iceless refrigerator" in the field, especially in carrying water. The celluloid "iceless refrigerator" described above is the most convenient, dependable and labor-saving device which the writer has used thus far for field inoculations with forest tree rusts.

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THE INFLUENCE OF MINERAL NUTRITION ON THE REACTION OF SWEET-CORN SEEDLINGS TO PHYTOMONAS STEWARTI

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Several workers (1, 2, 6, 7, 8, 10) have observed that the reaction of certain plants to infectious agents may be altered by varying the mineral composition of the nutrient solution. In connection with experiments being conducted in this laboratory with bacterial wilt of maize, the causal agent of which is *Phytomonas stewarti* Bergey *et al.* (*Bacterium stewarti* E. F. S.), it seemed desirable to ascertain whether the host-parasite relationship of this disease could be modified by mineral nutrition. A study was undertaken, therefore, to determine the influence of nitrogen, phosphorus, and potassium nutrition on the reaction of sweet-corn seedlings to *P. stewarti*. The responses shown by the seedlings are reported in this paper.

MATERIALS AND METHODS

Sweet-corn seedlings of the variety Golden Bantam were grown in white quartz sand previously washed with tap water. Five seeds were placed in each 4-inch porous clay pot, covered with a layer of sand about one-half inch in depth, and kept moist with tap water. When the seedlings had reached a height of 3 or 4 cm., usually 8 days after planting, they were thinned to 3 seedlings in each pot. Twenty-five pots were started for each nutrient treatment, but at time of inoculation 5 pots in each treatment were eliminated. The pots, set in saucers, were so placed on a greenhouse bench that all were exposed to approximately the same environmental conditions.

Nutrient treatments were started 7 or 8 days after planting. Each pot received 100 cc. of nutrient solution 3 times a week. The composition of the solutions is given in table 1. The salt proportions in these solutions

TABLE 1.—*Composition of nutrient solutions used in the different nitrogen, phosphorus, and potassium experiments*

Stock solutions (0.5 molar)	Volume of stock solutions per liter of nutrient solution in experiments on		
	Nitrogen	Phosphorus	Potassium
	cc.	*cc.*	*cc.*
KH_2PO_4	6.3	.....	.....
$MgSO_4$	7.1	7.1	7.1
$Ca(NO_3)_2$	.....	2.9	2.9
$CaCl_2$	2.9	.....	.....
$(NH_4)_2SO_4$	.....	2.8	.....
$NH_4H_2PO_4$	.....	.....	5.6
NH_4NO_3	0–142.9	.....	.....
NaH_2PO_4	.....	0–161.3	0.7
K_2SO_4	.....	3.2	0–76.7

were based on solution $T_3R_2C_2$ of the Jones-Shive series (3). This solution was chosen because it had produced good growth of corn in preliminary tests. In addition to the salts listed in table 1, boron as H_3BO_3 and manganese as $MnSO_4 \cdot 2H_2O$ were added to all solutions in concentrations equivalent to 0.5 p.p.m. of each element. Sufficient $FeSO_4$ was added to prevent chlorosis from iron deficiency. No attempt was made to hold the osmotic concentration of the solutions constant. The concentrations of nitrogen, phosphorus, and potassium were varied in separate experiments. When the concentration of one of these elements was varied, the concentrations of the other two were held constant.

The seedlings were inoculated 2 or 3 days after the first application of nutrients. A virulent, single-colony isolate (B–1011), described by McNew (5), was used as the stock culture. The culture was maintained on nutrient-dextrose agar slants at 20° C. and subinoculated into 200 cc. of nutrient broth (Difco), which had previously been supplemented with 0.5 per cent dextrose and adjusted to a pH of 6.8 to 7.0. After incubating for at least 72 hours, the culture was injected into the crown and leaf whorl of 30 seedlings in each group. The 30 seedlings remaining in each group were similarly injected with the sterile filtrate obtained by twice filtering the culture through Berkefeld "W" cylinders. These injections subjected all seedlings to the same inoculation wounding and to such metabolic by-products of the bacterium as might be present in the culture.

About 10 days after inoculation the total number of leaves, the number of killed leaves, and the number of necrotic lesions on each plant were recorded. The severity of infection was expressed as an infection index, based upon the average number of necrotic lesions per leaf. Since some leaves collapsed from a diffuse wilt or a coalescence of lesions, an accurate count of the number of necrotic lesions on these collapsed leaves was impossible. Each collapsed leaf, therefore, was arbitrarily classified as having 3 lesions. This calculated number of lesions on the collapsed leaves was added to the number of lesions actually recorded and the sum divided by the total number of leaves. The quotient so obtained was designated as the infection index.

After 18 or 20 days of nutrient treatment, the green weights of leaves and stems were determined. Dry weights were determined after the samples had been dried to constant weight at 100° C. in an electric oven.

Each nitrogen, phosphorus, and potassium experiment was carried out independently, since sufficient greenhouse space was not available to conduct more than one at a time. Therefore, the results of one experiment can not be directly compared with those of another, because of possible changes due to environmental factors. However, the experiments were all carried out in the same manner and for approximately the same duration of time, the nitrogen and phosphorus experiments in November and the potassium experiment in the preceding September. Each experiment was repeated at least once, with compatible results.

EXPERIMENTAL RESULTS

Influence of Nitrogen Supply on Host Reaction

The influence of variations in the nitrogen supply on the reaction of corn seedlings to *Phytomonas stewarti* was studied in the first experiment. The basic nutrient solution is described in table 1. Nitrogen was added as NH_4NO_3 in amounts ranging from 0 to 200 mg. of nitrogen per 100 cc. of solution. In all treatments the phosphorus concentration was held constant at 12.5 mg. and the potassium concentration at 10.0 mg. per 100 cc. of solution.

From the experimental data recorded in table 2, it is obvious that nitrogen

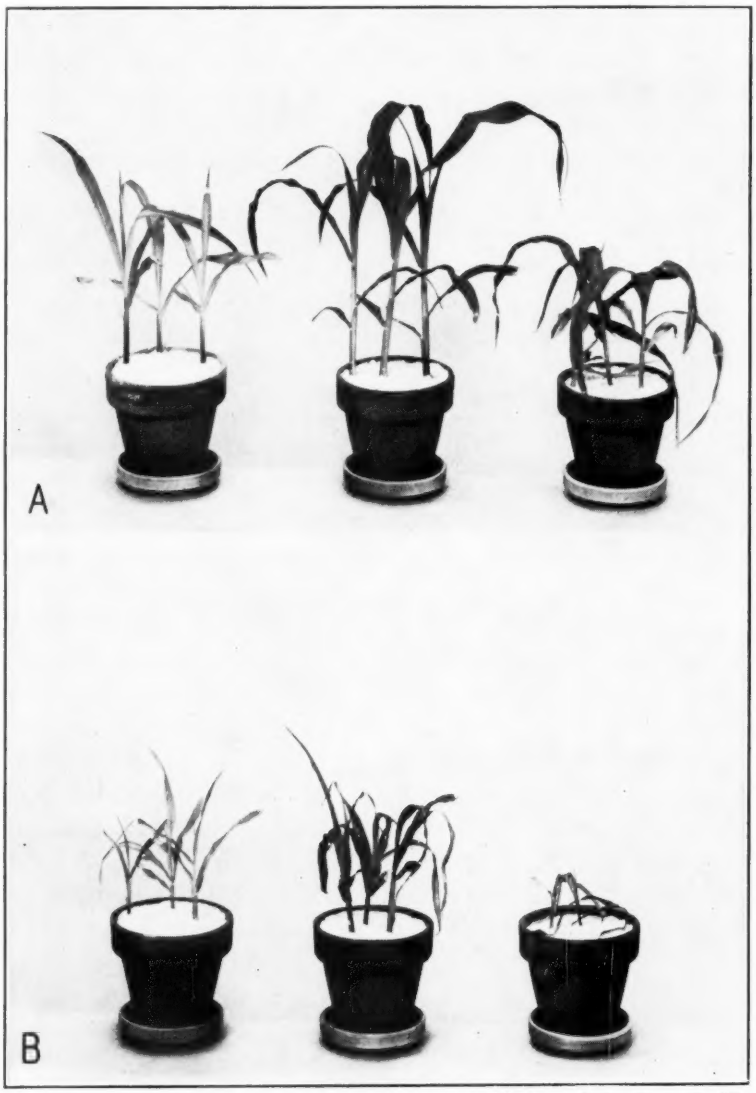
TABLE 2.—*Influence of nitrogen supply on growth and reaction of corn seedlings to infection with Phytomonas stewarti*

Nitrogen added	Average dry weight per plant		Reaction of diseased seedlings				
			Number of leaves			Number of lesions	Index of infection
	Healthy	Infected	Total	Invaded			
				Living	Dead		
mg./100 cc.	*gm.*	*gm.*					
0	.19	.16	124	29	1	47	.40
10	.40	.28	141	57	1	79	.58
20	.43	.23	119	46	5	63	.66
30	.45	.19	140	61	2	93	.71
40	.42	.25	123	65	5	108	1.00
70	.36	.23	131	58	5	109	.95
100	.32	.17	127	70	6	113	1.03
150	.28	.15	117	62	15	106	1.29
200	.19	.10	109	57	15	92	1.26

supply influenced very markedly the growth of corn seedlings in sand. Relatively low nitrogen levels (20–30 mg.) favored the maximum growth of seedlings. As the nitrogen level was raised above 30 mg., the seedlings made only limited growth. Representative seedlings of 3 different treatments were photographed 10 days after inoculation with the sterile filtrate of the culture. These seedlings, shown in figure 1, A, had received 0, 40, and 200 mg. of nitrogen, respectively, 3 times each week. The seedlings that had received no nitrogen were light yellowish-green and made very little growth. The lower leaves became shriveled and dry. Slight injury, due unquestionably to nitrogen toxicity, was noticeable in seedlings that had received 200 mg. of nitrogen 3 times each week. This injury was apparent as a wilting at the tips of older leaves. Some of these leaves later became entirely flaccid.

The data in table 2 on the reaction of the seedlings to the disease show that infection became more severe as the concentration of nitrogen increased. When more than 150 mg. of nitrogen were added per 100 cc. of solution, severity of infection remained fairly constant. Figure 1, B, shows infected seedlings of the same age and of the same nutrient treatment as those in figure 1, A. In the absence of nitrogen, seedlings showed primary invasion, but little or no secondary invasion. The necrotic lesions produced were small

and few in number. None of the nitrogen-deficient seedlings wilted. As the nitrogen level of the nutrient solution was raised, the pathogen produced larger and more numerous necrotic lesions, and caused a wilted condition of many of the invaded leaves. At the higher nitrogen levels, this type of



Photograph by J. A. Carlile

FIG. 1. Typical corn seedlings that had received 0, 40, and 200 mg. of nitrogen, respectively, 3 times each week, photographed after 2 weeks of nutrient treatment. A. Healthy seedlings. B. Infected seedlings 10 days after inoculation.

wilting was so intense that about one-half of the inoculated seedlings died within 2 weeks after inoculation. Thus, it is evident that infection was more severe at high nitrogen levels than at levels more favorable for plant growth. The possible significance of these results will be discussed later.

Influence of Phosphorus Supply on Host Reaction

In the second experiment, the influence of variations in the phosphorus supply was studied. The phosphorus levels of the solutions ranged from 0 to 250 mg. per 100 cc. of solution. The composition of the basic solution is given in table 1. The required amounts of phosphorus were added as NaH_2PO_4. In all solutions the nitrogen concentration was held constant at 8 mg. and the potassium concentration at 12.5 mg. per 100 cc. of solution.

The experimental data on the relation between phosphorus supply and growth of corn seedlings are given in table 3. These data show that the

TABLE 3.—*Influence of phosphorus supply on growth and reaction of corn seedlings to infection with Phytomonas stewarti*

Phosphorus added	Average dry weight per plant		Reaction of diseased seedlings				
			Number of leaves			Number of lesions	Index of infection
	Healthy	Infected	Total	Invaded			
				Living	Dead		
mg./100 cc.	*gm.*	*gm.*					
0	.34	.25	118	60	0	96	.81
20	.41	.25	115	68	2	109	1.00
40	.39	.32	122	73	1	121	1.02
60	.42	.29	121	69	2	125	1.08
80	.35	.28	117	60	3	123	1.13
100	.33	.25	113	66	4	126	1.22
150	.32	.24	113	67	5	116	1.16
200	.28	.19	110	68	4	128	1.27
250	.26	.17	103	63	8	121	1.41

plants grew poorly when more than 60 mg. of phosphorus were added 3 times each week. Maximum growth of seedlings was obtained when 20 to 60 mg. were added 3 times each week. Figure 2, A, shows pots of representative seedlings that had received 0, 100, and 200 mg. of phosphorus, respectively, 3 times each week for 3 weeks. Seedlings were injured somewhat by solutions containing either a deficiency or an excess of phosphorus. The seedlings that had received a solution deficient in phosphorus were purplish, with some yellowing apparent on a few of the lower leaves. These seedlings were slightly smaller than those that had received small amounts of phosphorus. The seedlings that had received the largest amounts of phosphorus grew poorly, but the only noticeable injury was a wilting at the tips of the lower leaves.

Figure 2, B, shows infected seedlings, on the same nutrient treatments as those in figure 2, A. At low phosphorus levels infection was characterized by the formation of definite necrotic lesions. At the higher phosphorus levels infection was manifested not only by necrotic lesions but also by dwarfing of the seedlings and a general wilting of the invaded leaves. This wilting was not intensified to the extent that death resulted within 2 weeks following inoculation, as was the case with the high-nitrogen seedlings. The infection gradually became more severe with increasing amounts of phosphorus, even though these high levels of phosphorus retarded seedling growth.

Since phosphorus was added as NaH_2PO_4, the sodium concentration increased simultaneously with that of phosphorus. In order to determine whether the increased severity of wilt at high levels of NaH_2PO_4 was due to phosphorus or to sodium, an experiment was carried out in which the sodium

Photograph by J. A. Carlile

FIG. 2. Typical corn seedlings that had received 0, 100, and 200 mg. of phosphorus, respectively, 3 times each week, photographed after 3 weeks of nutrient treatment. A. Healthy seedlings. B. Infected seedlings 16 days after inoculation.

concentration was held constant at all phosphorus levels by adding the required amounts of sodium as Na_2SO_4. The results from this experiment were comparable with those recorded in table 3, in that infection was more severe at the higher levels of phosphorus. However, seedlings that received

sodium but no phosphorus were somewhat more severely infected than those that received a deficiency of both sodium and phosphorus. These findings indicate that the differences in the host-parasite association brought about by the addition of increasing amounts of NaH_2PO_4 were due primarily to the variations in the phosphorus levels and not to the variations in sodium levels.

Influence of Potassium Supply on Host Reaction

In the third experiment, the influence of potassium supply was studied. The composition of the basic solution is given in table 1. Potassium was added as K_2SO_4 in amounts ranging from 0 to 300 mg. per 100 cc. of solution. In all solutions the nitrogen concentration was held constant at 8 mg. and the phosphorus at 10 mg. per 100 cc. of solution.

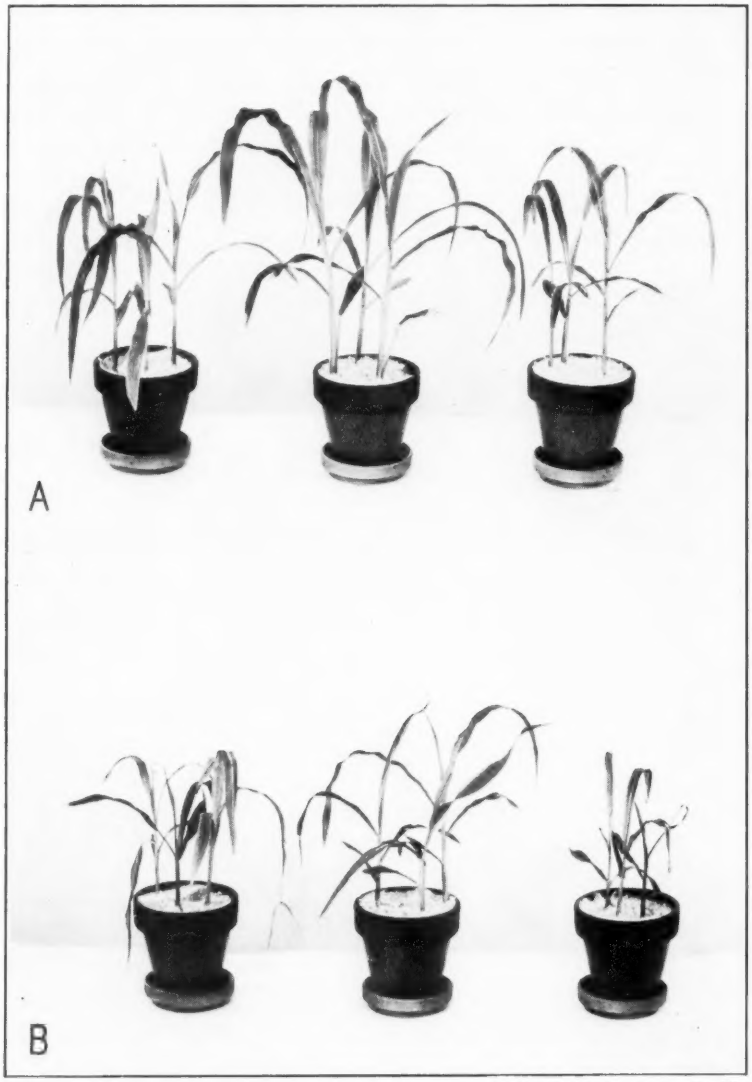
The experimental data given in table 4 show that growth of the healthy

TABLE 4.—*Influence on potassium supply on growth and reaction of corn seedlings to infection with Phytomonas stewarti*

Potassium added	Average dry weight per plant		Reaction of diseased seedlings				
			Number of leaves			Number of lesions	Index of infection
	Healthy	Infected	Total	Invaded			
				Living	Dead		
mg./100 cc.	*gm.*	*gm.*					
0	.44	.36	172	98	13	194	1.35
20	.56	.45	172	82	10	134	.95
40	.61	.39	170	66	14	105	.86
60	.67	.37	165	70	16	122	1.03
80	.59	.43	174	80	12	143	1.03
100	.61	.39	162	69	18	119	1.07
150	.58	.43	167	74	16	135	1.10
200	.49	.30	143	67	27	121	1.41
250	.47	.34	144	63	27	108	1.31
300	.41	.26	131	56	17	94	1.11

seedlings increased as the concentration of potassium increased to a level of 60 mg. per 100 cc. of solution. Growth was retarded as the concentration was increased beyond this point. Infected seedlings that had received between 20 and 150 mg. of potassium per 100 cc. of solution showed little variation in growth. Figure 3 shows representative healthy and infected seedlings that had received 0, 80, and 200 mg. of potassium 3 times each week for 3 weeks.

Plants that received no potassium showed distinct symptoms of potassium deficiency. The older leaves of healthy plants showed a necrosis, which spread from the tip along the margins to the leaf sheath. The young leaves of healthy seedlings were apparently normal, whereas the young leaves of infected seedlings showed a very marked chlorosis. Definite symptoms of toxicity appeared on all seedlings that had received high levels of potassium. The young whorls failed to develop normally, and they gradually became necrotic. The older leaves became light brown and necrotic. In all treat-



Photograph by J. A. Carlile

FIG. 3. Typical corn seedlings that had received 0, 80, and 200 mg. of potassium, respectively, 3 times each week, photographed after 3 weeks of treatment. A. Healthy seedlings. B. Infected seedlings 16 days after inoculation.

ments where toxicity occurred, the toxicity symptoms were more severe on the infected seedlings than on the healthy seedlings. When 200 mg. of potassium were added 3 times each week, irregular blotches developed on leaves of the infected seedlings but not on those of the healthy seedlings. The same condition with regard to necrosis of the leaf whorls was noticeable at the next higher potassium level (250 mg.). At the highest potassium level (300 mg.), more leaf whorls were injured in infected seedlings than in healthy seedlings.

The data on disease response show that potassium-deficient seedlings were very severely infected, whereas seedlings that had received 20 or 40 mg. of potassium per 100 cc. of solution were only slightly infected. However, the infection became more severe as the potassium level was raised from 40 mg. to 200 mg. These findings with regard to potassium deficiency are very striking and are apparently significant, since 2 subsequent repetitions of this experiment revealed the same relationship. The type of necrotic lesions formed as the result of inoculation appears to be similar in all treatments. Wilting of invaded leaves was more prevalent at the higher potassium levels, but none of the seedlings died as a result of infection in the 14-day period following inoculation.

DISCUSSION

The average number of necrotic lesions per leaf should serve as a satisfactory measure of the host reaction of the pathogen, since corresponding host tissues in all treatments were exposed to the bacterial culture. The infection index, as used in the experiments reported above, is an approximate measure of the number of leaf veins that necrose because of invasion by *Phytomonas stewarti*. Since the severity of infection usually was noted 10 days after inoculation, the infection index, as used here, is essentially a measure of primary infection. Seedlings with low infection indexes suffered very little secondary invasion and generally recovered.

It has been reported frequently that an actively growing host is more susceptible to disease than a slow-growing host. Trelease and Trelease (9) have shown, however, that some condition, or conditions, apparently independent of host vigor appears to determine the susceptibility of wheat to mildew (*Erysiphe graminis* DC.). A similar situation seems to exist in the host-parasite complex of bacterial wilt of maize, as shown by the experimental evidence here reported. For example, seedlings that received nitrogen or phosphorus at concentrations optimum for plant growth were more severely infected than those deprived of the element in question. On the other hand, seedlings that received small amounts of potassium grew better but were less severely infected than those deficient in potassium.

An increase in the concentration of nitrogen, phosphorus, or potassium beyond that optimum for plant growth, as judged by dry weight, increased the severity of the disease. It is probable that the excessive wilting produced by these treatments is due partly to high osmotic pressure of the solution and partly to invasion by the bacteria. The quantity of water available for the leaves apparently is reduced not only by a plugging of the tracheal tubes by the organism, but also by the increased osmotic concentration of the solution.

The effect produced by different amounts of nitrogen, phosphorus, or potassium on the reaction of corn seedlings to *Phytomonas stewarti* may be due to a change in (1) the composition of the host cell so as to alter its susceptibility to parasitic attack, (2) the rate of multiplication of the pathogen, or (3) the virulence of the pathogen within the host. From the experimental

evidence presented in this paper, it is not known which of these changes has been instrumental in producing the observed reaction.

If the nutrient solution affected the pathogen directly, the action would undoubtedly be brought about through a change in the composition of the transpiration stream. Lowry, Huggins, and Forrest (4) have shown that the mineral composition of exuded maize sap depended to some extent on the fertilizer treatment. If the nutrients added in the experiments reported here were taken up by the roots and transported through the tracheal tubes, it is evident that the pathogen was growing in a medium the composition of which varied with that of the nutrient solution. If there is a difference in the composition of tracheal sap in seedlings grown under different nutrient treatments, it might change the growth rate of the bacteria or encourage the differential development of strains that vary in pathogenicity.

It is possible that competition between host and pathogen for the available nutrients may be a factor governing the severity of the disease. When the element in question is present in low concentration, the normal processes of the host cell may utilize it to such an extent as to leave an actual deficiency of it as far as the pathogen is concerned. As the supply of the element increases, the competition between host and pathogen may develop sufficiently to overcome any resistance the host cell might normally have possessed. With high-phosphorus and high-potassium nutrition, a dynamic equilibrium apparently exists between host and pathogen. With high-nitrogen nutrition, however, the pathogen was able to kill the host.

It is of interest to note the response shown by potassium-deficient seedlings to the disease. The infected seedlings developed a pronounced chlorosis, a condition not shown by the healthy seedlings. Moreover, the disease was more severe in seedlings deficient in potassium than in those supplied with low concentrations of potassium. This difference in severity may possibly be explained as follows: The pathogen may have a low potassium requirement and may cause considerable injury when inoculated into seedlings weakened by a deficiency of potassium. However, with the addition of small amounts of potassium, the seedlings start normal growth, and in so doing may become more resistant.

SUMMARY

The influence of nitrogen, phosphorus, and potassium nutrition on the reaction of sweet-corn seedlings to *Phytomonas stewarti* was studied. Severity of infection was noted 10 days after inoculation.

Seedlings dwarfed by high concentrations of nitrogen, phosphorus, or potassium were more severely infected than those grown at concentrations more conducive to rapid growth. Seedlings deficient in either nitrogen or phosphorus were only slightly infected, whereas potassium-deficient seedlings were severely infected.

Seedlings supplied with a nitrogen-deficient solution developed small necrotic lesions but little or no wilting of invaded leaves. With high nitro-

gen, wilting was so intense that about one-half of the seedlings died within 2 weeks after inoculation.

At low phosphorus levels, infection was characterized by the formation of definite necrotic lesions, whereas at high levels infection was manifested not only by necrotic lesions but also by a dwarfing of the seedlings and a general wilting of the invaded leaves. None of the seedlings died as a result of infection.

In the potassium-deficient seedlings, infection was more severe than in seedlings receiving small amounts of potassium. The potassium level had no effect on the type of necrotic lesions formed, but high levels favored the wilting condition of invaded leaves.

It is evident, therefore, that mineral nutrition exerts some influence on the host-pathogen complex of bacterial wilt of maize. The exact changes brought about in this complex by nutrition have yet to be ascertained.

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PHYTOPATHOLOGICAL NOTES

Lightning Injury in Banana Plantations.—Spots of killed bananas, that may be from 40 to 60 feet in diameter, have been noted in large banana plantations. These areas appear after severe electric storms, and it is believed that they could be caused only by lightning. Detailed examination and study were made of 3 spots in banana plantations in the vicinity of Tela, Honduras. Two of these were observed, respectively, on September 24, 1923, and October 1, 1923, and the last one was examined on July 28, 1930. The first two spots were undoubtedly produced during a severe electric storm of September 16, 1923. The last spot observed must have resulted from a storm that occurred some 2 weeks earlier. The symptoms as described were the same in each of these areas.

The affected spots are characterized by a twisted mass of collapsed banana plants in the center of the areas and partially cooked plants that had yellowed, drooping leaves on the outer edges of the areas (Fig. 1). The outer row of plants facing the center of the area was not wholly affected; only the sides of the plants toward the center of the spot appeared scorched or had yellowed leaves. Some low-growing weeds were scorched, but most of them apparently were still in a normal state of growth. No burning was noted in the soil refuse nor was any apparent change in the soil observed.

A detailed examination of the worst affected plants in the center of the spots showed them to be entirely wilted down, as if they had been scalded. The plants had collapsed in a twisted, cooked mass. The interior of the pseudostems had the appearance of tissues that were immersed in boiling water. The entire pseudostems of the worst affected plants had a cooked appearance that extended down to the rhizomes at the ground surface. No evidence of rot was present at this early stage. The smaller suckers arising from these rhizomes were for the most part not severely affected and the rhizomes appeared to be normal. A later examination of the lightning-struck areas showed this to be true, as the suckers grew and produced normal plants, thereby filling the affected area again with healthy plants.

Plants at the outer edges of the spots were less affected and were characterized by a scorching and falling over of the upper leaves of the worst affected plants, or merely a yellowing and drooping of the leaves of plants on the extreme outer edges of the spot. The outer leaf sheaths of the most severely affected plants were slightly browned and appeared as if scalded. The young central group of developing leaves within the pseudostem of these were dried, pithy and white. This interior symptom ran through the pseudostem to the rhizome.—Otto A. Reinking, New York State Agricultural Experiment Station, Geneva, New York.

Control of Sclerotinia and Botrytis Stem Rots of Greenhouse Tomatoes and Cucumbers.—Sclerotinia and Botrytis stem rots, Botrytis fruit rots, and

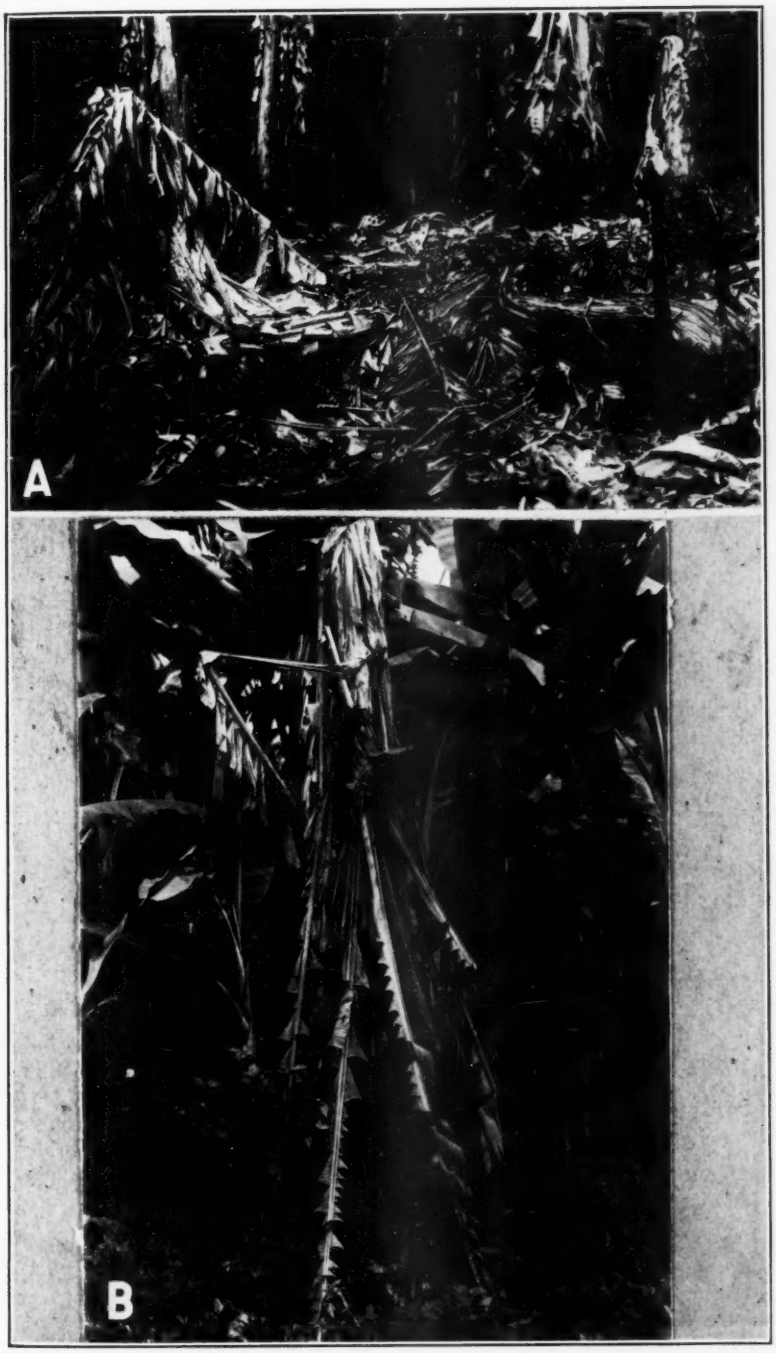

FIG. 1. Lightning injury. A. Collapsed banana plants in center of affected area. The mass of softened, twisted pseudostems is characteristic. Photographed on Sept. 24, 1923. B. Partially cooked banana plant at edge of affected area. Photographed on Oct. 1, 1923.

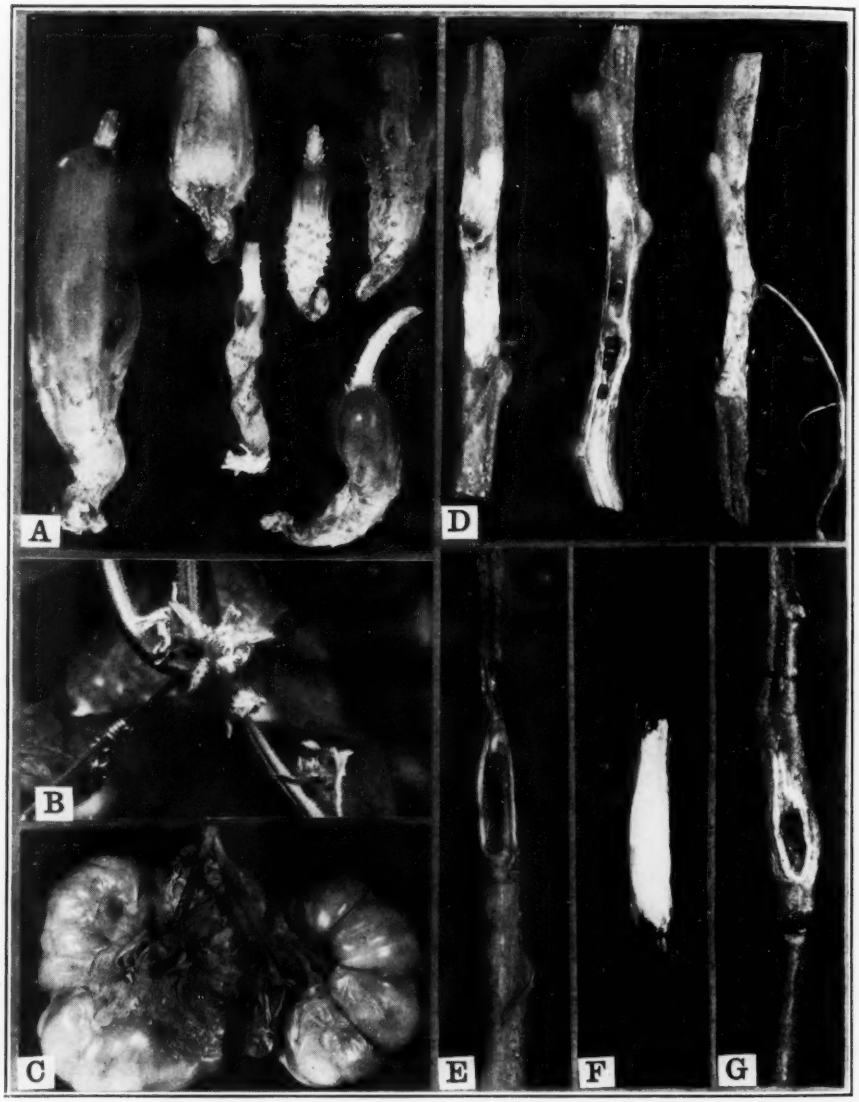

FIG. 1. A. Botrytis rot of cucumber fruit. In most instances this phase of the disease appears to have its origin from blossoms which were fertilized before coming infected. B. Blossom blight of cucumber caused by Botrytis Sp. This is a rather common stage of the disease, especially on male flowers. C. Botrytis stem end rot of tomato. Under favorable conditions losses from fruit infections may be large. D. Tomato stems infected with *Sclerotinia libertiana*. Note leaf petiole on right of picture through which the fungus entered the stem from the ground. The figure on the left became infected at point where leaf had been removed. The sclerotia in the center figure are typical. A similar disease usually without sclerotia, although small ones are sometimes found, may be caused by a *Botrytis Sp.* As a rule, an abundance of spores are visible when the disease is caused by Botrytis. Quite often the two fungi are found in the same lesion. E. Tomato stem prepared to receive the Bordeaux paste treatment. This stem was infected by Botrytis. F. The same stem after Bordeaux paste had been applied. G. Another stem, similarly diseased and treated, with Bordeaux removed one month after treatment was applied. Stems nearly rotted through have been saved by the Bordeaux paste treatment.

blossom blight of greenhouse tomatoes and cucumbers are diseases usually considered to be of minor importance. (Fig. 1, A–D.) Occasionally, however, these troubles are of genuine commercial concern. For 2 months of the spring of 1935 damp, cloudy weather prevailed in the Chicago area and, as a result, the above diseases became so well established in a few greenhouses of tomatoes and cucumbers as to cause losses amounting to 60–75 per cent. It was estimated that total losses in Cook County averaged between 15 and 20 per cent. Although these diseases are seldom of any such importance, if they do occur, the control measures now recommended (soil sterilization and spraying plants with 4–4–50 Bordeaux mixture) are of doubtful value in saving an infected planting. We have observed that soil sterilization, although effective for the control of stem rot (timber rot), caused by *Sclerotinia libertiana* Fckl., will not prevent Botrytis fruit and stem rot from becoming quickly established in new plantings. The latter disease is usually in evidence whenever the environment and crops are suitable for its development. As noted below, spraying has been effective in a few instances.

With the above facts in mind, several methods and materials were tested with the hope of finding a suitable control for both troubles—one that could be applied effectively after the diseases had made their appearance.

Experiments were conducted in cooperation with several commercial growers and also at the University under controlled conditions. To date nothing outstandingly effective has been found for the control of Botrytis blossom blight and fruit rot on either tomatoes or cucumbers. In a few tests sulphur sprays and dusts appeared effective on both crops, as did also basic copper sulphate and Bordeaux mixture sprays. Subsequent tests, however, indicate that these treatments are not to be relied upon; in fact, the Bordeaux spray is almost sure to injure cucumbers. Fortunately, the fruit rot and blossom blight phases of the Botrytis disease are not the most important. The greatest losses are sustained from stem rots caused by the two fungi.

Of the several different methods tried as possible control measures, a Bordeaux mixture made of 1 part copper sulphate, 2 parts lime, and enough water to make a thick paste, has given nearly perfect control of stem rots after they had become well established. The most effective method of application is to gouge out the infected tissue with a knife or stick and fill the cavity thus formed with the paste. (Fig. 1, E–G.) Plants nearly rotted through, when thus treated, have been saved and continued to develop and produce apparently normal crops. The treatment has been equally effective on both tomatoes and cucumbers, but, in a few instances, it has caused injury to cucumbers, resulting in the yellowing of some of the leaves.—K. J. KADOW, H. W. ANDERSON, and S. L. HOPPERSTEAD, Department of Horticulture, University of Illinois, Urbana, Illinois.

A Root and Collar Disease of Pine Seedlings Caused by Sphaeropsis ellisii.—During the investigation of *Phytophthora cinnamomi* root disease of *Pinus resinosa* seedlings, many of the 3- to 5-year-old trees in two Maryland nurser-

ies were found to be dying from a root rot characterized by symptoms differing from those typical of Phytophthora rot. Resembling Phytophthora rot to the extent that the area attacked often extended from the collar to a point below the soil line, this rot was, however, not characterized by exudation of resin nor by the infiltration of resin in the wood, which commonly follows *Phytophthora* attack on this host. The invaded bark tissue was deep red, with black streaks continuing into the xylem and often throughout the entire stele. Five- or 6-year-old *Pinus strobus* trees, showing similar symptoms, were received from a plantation in Wisconsin.

A fungus, black in culture, was isolated from the discolored tissue of both the *Pinus resinosa* and the *P. strobus* trees showing the symptoms mentioned. Sections of infected root and collar tissue were placed in a damp chamber, where typical *Sphaeropsis* pycnidia and spores (averaging 36 x 12 μ) developed within a few days. Neil E. Stevens examined the fungus, both in culture and on the host, and identified it as *Sphaeropsis ellisii* (Sacc.). *S. ellisii* has been previously reported as a common parasite on a large number of coniferous hosts, but always as the cause of various twig die-back diseases.

Single-spore isolations of the fungus from *Pinus resinosa* were used in inoculations in comparison with isolates of *P. cinnamomi* obtained from diseased roots of seedlings of the same species and considered truly pathogenic on them.[1] Inoculations were made by inserting rice grains on which the fungus was growing in cuts extending through the bark to the cambium of 3-year-old *P. resinosa* seedlings. Check inoculations were made by inoculating with sterile rice and with cultures of *Sclerotium bataticola* and *Pestalozzia funerea*, also isolated from diseased *P. resinosa* roots. Immediately after it was inoculated, the wound was covered with dry cotton and linen tape.

In the first test half of the seedlings were inoculated at the soil line and the remainder an inch below it. Within 20 days the 12 seedlings inoculated with *Sphaeropsis*, the 40 inoculated with 2 lines of *Phytophthora*, and 5 of the 20 inoculated with *Pestalozzia* had died, whereas the 8 inoculated with *Sclerotium* and 20 with sterile rice remained healthy. The place of inoculation apparently did not influence the result, since rot of both root and collar resulted from each.

In a second test 6 trees each were inoculated at the collar with cultures of *Phytophthora*, *Sphaeropsis*, and *Pestalozzia*. Bits of rice on which *Phytophthora* and *Pestalozzia* were growing were inoculated into small cuts, while the rice on which *Sphaeropsis* was growing was merely placed in contact with the nonwounded bark. Inoculations with sterile rice were used as a check. Over a period of 18 days, during which *P. cinnamomi* invaded tissue an average of 7.5 cm. in each direction from the collar, the isolate of *S. ellisii* progressed an average of 5.5 cm. down into the roots and 12 cm. up the stem. In both cases all inoculated seedlings were killed. Three of the 6 seedlings inoculated with *Pestalozzia* were killed, with the fungus apparently present up to 8 cm. in the stem but not in the roots at all. All checks remained

[1] Jackson, L. W. R., and B. S. Crandall. A Phytophthora root and collar rot of *Pinus resinosa* seedlings. (Abstract) Phytopath. 25: 22. 1935.

healthy. In all the *Sphaeropsis* and *Phytophthora* inoculations, symptoms were produced, typical of those found associated with the respective fungi in the field and the fungus reisolated.—BOWEN S. CRANDALL, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

The Propagation of Tobacco Plants from Root Cuttings.—During the summer of 1936 a crop of tobacco (*Nicotiana tabacum*) of the variety "Virginian" was grown in the experimental garden of the Virus Research Station here. The plants were infected with a strain of Tobacco Virus No. 1 kindly supplied by the Department of Animal and Plant Pathology of the Rockefeller Institute, Princeton, N. J. They were harvested in October, 1936, the ground being dug over and narcissus bulbs planted in connection with an-

FIG. 1. Fragment of the root of an old tobacco plant from which has grown a new plant.

other investigation. At the end of July, this year, it was noticed that here and there on the plot plants of tobacco had made their appearance, the foliage of the bulbs having died down. The tobacco leaves all showed symptoms of ordinary tobacco mosaic (Tobacco Virus No. 1). Since there is little sound evidence that this virus is transmitted in the seed, a careful examination of

the diseased plants was made to discover if they were in fact seedlings infected through the seed. When the plants were dug up it was found that they had developed from portions of roots inadvertently left in the ground when it had been cleared in the previous autumn. It would appear that the tobacco plant can readily propagate itself by root cuttings and that under mild climatic conditions, at least, this may constitute a possible source of infection for the next year's crop. The question of the persistence or not of the virus in leaves and plant débris may be of less importance under these conditions than the possibility of the appearance of actual plants from portions of diseased roots of the previous crop, analogous to the "Groundkeepers" found in potato fields.

Figure 1 shows the appearance of one of the roots with its attached plant. —JOHN CALDWELL and A. L. JAMES, Department of Botany, University College, Exeter.

NOTICE TO MEMBERS OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY CONCERNING DESCRIPTION AND NOMENCLATURE OF PLANT VIRUSES

It was voted at Indianapolis to have as a special item on the next annual program of the Society a discussion of the description and nomenclature of plant viruses. The Council has taken the following action looking toward the development of this important feature of the program.

The following committee has been appointed to arrange for the virus program at Richmond, Virginia: James Johnson, Chairman; L. O. Kunkel, C. W. Bennett, Eubanks Carsner, H. H. McKinney, E. S. Shultz, W. D. Valleau.

The following resolution, passed by the Council, will be presented to the Society for its consideration at Richmond:

The Council recommends to the Society for its favorable consideration at the Richmond meeting the following: Resolved that the American Phytopathological Society express to the International Committee on Description and Nomenclature of Plant Viruses appreciation of the work they have done and confidence in the plan outlined in their "Illustration of Proposed System of Nomenclature for Plant Viruses," which was presented in mimeographed form at the Sixth International Botanical Congress (Amsterdam, 1935) and adopted in principle, the committee being empowered to continue its considerations and establish an acceptable system of virus nomenclature. It is further the sense of this Society that it is desirable for individual workers to cooperate as much as possible with the International Committee on Descriptions and Nomenclature of Plant Viruses and in so far as feasible to refrain from adopting any new system of nomenclature until this committee has had the opportunity to make its next report.

Drs. Johnson and Kunkel extend to all who are interested in the description and nomenclature of plant viruses a cordial invitation to make criticisms

or suggestions for the betterment of the plan[1] now under consideration by the International Committee, which will be the basis for the discussion at Richmond.

R. S. KIRBY, Secretary.

BOOK REVIEW

KENNETH M. SMITH, D.SC., PH.D. *A Textbook of Plant Virus Diseases.* Pp. 615, 101 figs. London. J. and A. Churchill, Ltd. 1937. 21 s.

This book is the outcome of a serious effort to bring together in a handy volume the scattered world literature on virus diseases of plants. The author himself has been occupied for about 18 years with all the phases of plant virus work. In his earlier book: "*Recent Advances in the Study of Plant Viruses*" he has given an excellent review of problems and methods, together with a short survey of special viroses. The new book is entirely devoted to the special treatment of plant viroses. It is an extensive compilation and must have entailed a vast amount of work. There is no other book of its kind in existence. The reviewer does not like the title. It is not a text-book but a reference work. It is really a kind of dictionary of plant viruses with descriptions of the various diseases they cause. Nor does the reviewer agree with the author's attitude in wanting to make a special science of "Virology." We have already enough water-tight compartments; the study of viroses of plants is the last that can be circumscribed. Already, it needs botanists, entomologists, biochemists, etc! Surely, it is sufficient to include it in Plant Pathology.

The scheme of classification followed is a modification of and an improvement on that suggested by James Johnson, of Wisconsin. Each virus is grouped according to its first-described or most important host plant, and the hosts are arranged according to the families to which they belong. Under each particular host plant, for which the Latin generic name is used, the viruses are numbered consecutively 1, 2, 3, etc., and the strains of certain viruses are denoted 1a, 1b, 1c, etc. The properties of each virus *in vitro* (so far as they are known at present), its mode of transmission, and the diseases it causes are described. The insect vectors are treated in some detail in a separate section of the book.

Special mention should be made of the appendix, giving the most characteristic symptoms of the various virus diseases on the more important host plants. It helps the student to arrive at a preliminary diagnosis of a suspected virus disease and also enables him, by means of the page references, to find in the book the information on all the viruses that attack any one particular plant. By this means, also, the disadvantage of the classification of the viruses, rather than the diseases they produce, will be partly overcome.

In many respects this book is excellent. Its style is clear, and it will be of value for many research workers and teachers of plant pathology. It is well illustrated, and printed on good paper. Some critical remarks must, however, be made and they will be restricted to Chapter VI, in which the viroses of the potato are treated in detail. Most of the descriptions are good, but the historical sequence of the discoveries shows some gaps. This may be due to the fact that the author, in spite of the existence of excellent abstracting journals, such as the Review of Applied Mycology and Biological Abstracts, has an incomplete knowledge of such literature as is not English or American.

In the introduction to Chapter VI, W. A. Orton, together with Quanjer and Appel, is considered the father of modern plant-virus work. In the same metaphorical language, however, Appel and Orton should really be regarded as godfathers. Appel stood godfather to the name "Blattrollkrankheit," Orton to the name "Potato Mosaic." But what is a name when it is not known whether the godchild is a fungal, bacterial, or non-parasitic disease?

What the author says of Solanum virus 1 Orton, sufficiently proves that it is not possible at this date to determine which virosis of the potato Orton had before him. Solanum virus 1 Orton is wrongly considered as being identical with Quanjer's aconecrosis virus and his interveinal mosaic virus. Köhler's ring mosaic virus is not considered at all. That the difference in severity of the symptoms produced by the strains

[1] Johnson, James. The nomenclature of plant viruses. Proc. Sixth International Bot. Cong. (Amsterdam) 2: 193–195. 1936.

of Solanum virus 1 would be less distinct on potatoes than on tobacco is not the view of the present reviewer. The preponderating English-American orientation of the author appears also from the fact that he appears to be unaware that only a restricted number of commercial potato stocks contain viruses of the "healthy potato virus" type on the continent of Europe.

Solanum virus 2 Orton is mistakenly considered to be synonymous with Atanasoff's stipple-streak virus and Quanjer's Acropetal Necrosis Virus. In contrast to Orton's Solanum virus 2 the virus described by Atanasoff and Quanjer produces internal necrosis in the tubers. Köhler's Strichelvirus is not mentioned in this connection. Solanum virus 2 is not a simple one but is rather an association of different viruses that must be distinguished.

Solanum virus 4 Murphy has a pathological effect that does not appear only as primary symptoms, for secondary symptoms also are known, to which Oortwijn Botjes first called attention.

Photomicrographs of the histopathological effect of viruses 1 and 2 are given; but an illustration of the classical phloem-necrosis, described in 1913 by Quanjer, is lacking. Although, in 1920, Oortwijn Botjes was the first to describe the transmission of Solanum virus 14 by the peach aphis, the name of K. M. Smith and the year 1929 alone are mentioned in this connection. Elze, in 1927 (*i.e.*, 4 years earlier than K. M. Smith), was the first to prove and describe the obligatory connection between this virus and its vector and to determinate the "waiting" time. Elze was also the first to give exact data on the transmission of this virus with true potato seed. Thung's fundamental work of 1928 on the physiological aspects of the leaf-roll disease is not mentioned.

In a book of this kind it is perhaps inevitable that omissions and errors will be detected, such as those just mentioned; but, though unfortunate, they do not detract unduly from the general usefulness of the text and they can be rectified in a future edition.—H. M. QUANJER, Instituut voor Phytopathologie, Wageningen, The Netherlands.